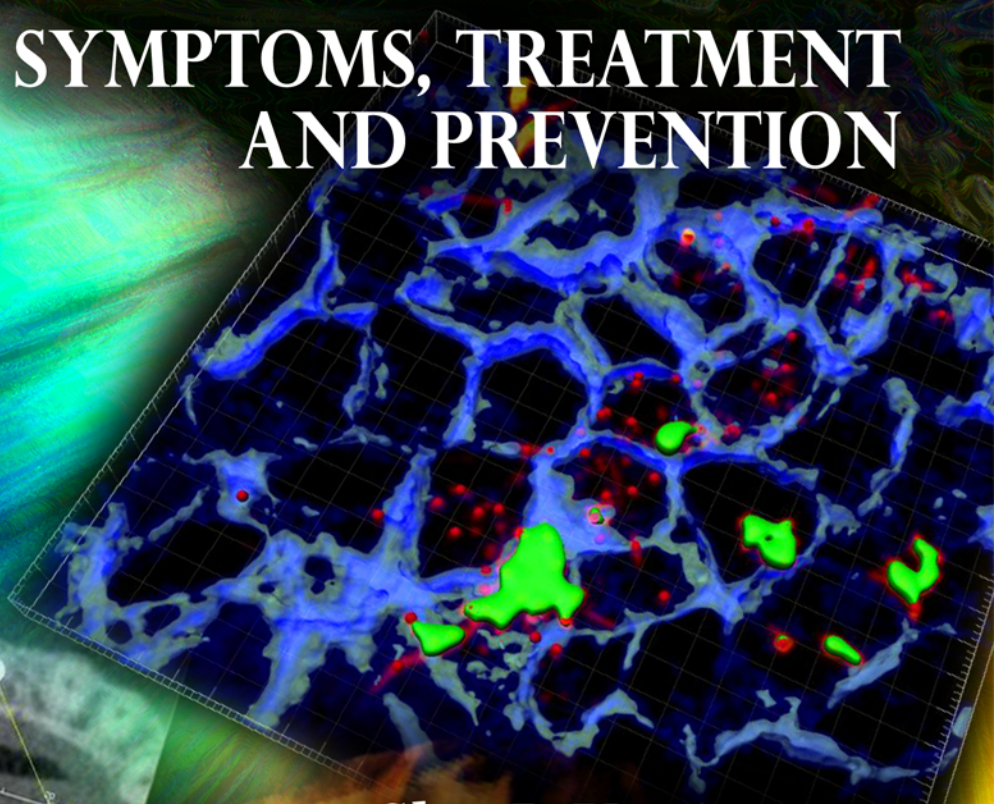
Periodontal Disease

Symptoms, Treatment and Prevention



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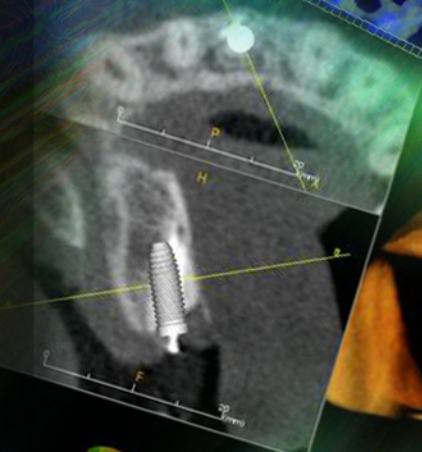

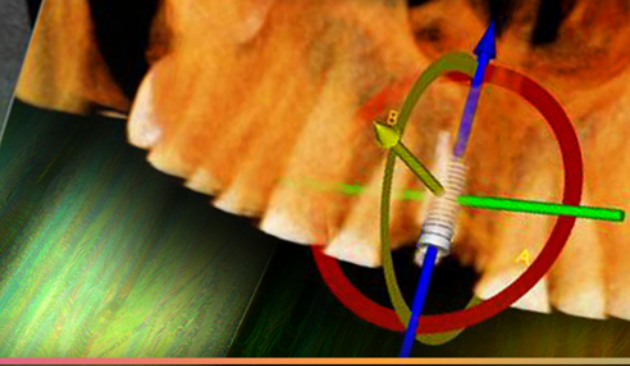

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PERIODONTAL DISEASE: SYMPTOMS, TREATMENT AND PREVENTION

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DENTAL SCIENCE, MATERIALS AND TECHNOLOGY

PERIODONTAL DISEASE: SYMPTOMS, TREATMENT AND PREVENTION

SHO L. YAMAMOTO
EDITOR

Nova Biomedical Books
New York

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Preface

Periodontal disease is a chronic bacterial infection characterized by persistent inflammation, connective tissue breakdown and alveolar bone destruction. The chronic inflammation associated with periodontal disease represents the host response to bacterial plaque, mediated by the environment in which the response occurs. This book presents topical research data in the study of periodontal disease, including aesthetic periodontal therapy and root coverage techniques; clinical features of periodontal diseases in children and adolescents; biomechanics and the perioprosthetic patient; maternal periodontitis and perinatal outcomes; identifying patients with enhanced disease susceptibility in periodontal disease; and inflammatory mediators and oxidative stress in periodontal disease.

Chapter I - Aesthetic considerations have influenced the management of dental maladies in varying degrees for many years. For many years the goals of periodontal surgery have been determined by functional aspects only. During recent years periodontal surgery has shifted its focus from achieving more functional goals toward a combination of both good functional and esthetic results. While accomplishing the best possible functional result, esthetics should not only be maintained, but also enhanced. Sometimes the esthetic outcome is the only important factor and function becomes secondary (e.g. treatment of recessions or the creation of papillae). Predictability becomes the key word in this type of periodontal surgery. Patient awareness and expectations have increased recently to the point that less than optimal esthetics is no longer an acceptable outcome. Periodontal plastic surgery would accordingly be defined as “surgical procedures performed to prevent or correct anatomic, developmental, traumatic or disease induced defects in the gingiva, alveolar mucosa or bone”. The present chapter is presenting and discussing the clinical outcomes of several root coverage techniques: pedicle soft tissue grafts, rotational flaps, coronally advanced flap, semilunar flap, free soft tissue graft, nonsubmerged grafts, submerged grafts etc.

Chapter II - The clinical features of periodontal diseases in children and adolescents differ from those in adults. Periodontitis is extremely rare in children, except those complicated with certain kinds of systemic diseases, whereas gingivitis is commonly encountered. Childhood gingivitis can be reversed by professional mechanical tooth cleaning in combination with tooth brushing instruction. On the other hand, gingivitis becomes increasingly prevalent with age through the adolescent period, and early diagnosis and appropriate interventions are necessary to prevent the onset of marginal periodontitis during adolescence. Since most children with periodontitis possess a background of abnormal immune responses, they have a lower likelihood of good prognosis,even though diligent interventions are performed. Other types of periodontal diseases include gingival recession,

which is mainly caused by traumatic occlusion, and gingival overgrowth, which has a hereditary background and is associated with specific medication such as antiepilepticphenytoin. In addition, cases with a rapid loss of gingival attachment and alveolar bone due to mechanical injury at the periodontal sulcus, termed “acute periodontitis,” are also encountered. Furthermore, an unintentional attachment loss, when materials such as small plastic tubes being fitted to the teeth are inserted, is a unique type of periodontitis in young children. It should be noted that periodontitis associated with anatomical anomalies, which are derived from fragile periodontal attachment, is also encountered.

Considering the etiology of periodontitis, it is important to identify periodontitis-related bacterial species, since the disease is generally known to be caused by specific bacteria. However, most of those belong to the obligate anaerobic group, and it is difficult and time-consuming to isolate them. On the other hand, recent developments in molecular biological techniques have enabled rapid identification of species using bacterial DNA extracted from various kinds of clinical specimens. Such approaches do not require isolation of viable bacteria and even small amounts of DNA can be detected using PCR techniques. With such modern techniques, the author have evaluated the distribution of periodontal bacterial species in children, changes of species in the same subjects over a long interval, combinations of species simultaneously detected, and mother-to-child transmission. In addition, the distributions of bacterial species in children with Down’s syndrome and other developmental disabilities have been analyzed. The authors’ results have provided valuable information regarding bacterial profiles in clinical specimens, which should lead to further beneficial methods for clinical use in the near future.

Chapter III - In advanced perioprosthetic cases where the periodontium’s integrity is severely compromised and the dental barrier’s function is extremely disrupted, the biomechanical response to the extrinsic mechanical stimuli of the system including the prosthetic restoration supported by the biological tissues is quite altered. The differentiated altered experience of the functional loading due to the lowered periodontium’s threshold along with the apical shift of the system fulcrum due to the periodontium’s structure reduction require a modified design of the restoration’s metal framework as a critical factor in the system’s survival in order to secure the expected longevity of both the restorative and biological structures, capturing the failure initiation of either progressive tissular or technical collapse. So, the purpose of the present study was to: a. analyze the way by which the periodontium reacts to the developing forces and how its integrity is related to the experience of the stress field on the perioprosthetic patient; b. determine the parameters defining the tooth prognosis in the perioprosthetic patient and how the restoration type is involved; c. report the clinical significance of tooth splinting by cantilever cross arch fixed partial denture applied on the perioprosthetic patient and the way it is related to the response of the reduced periodontium and finally d. investigate the clinical significance of the specific design of the metal framework in cantilever cross-arch fixed partial dentures via a theoretical finite element model.

Chapter IV - Periodontal disease is a chronic bacterial infection characterised by persistent inflammation, connective tissue breakdown and alveolar bone destruction. The chronic inflammation associated with periodontal disease represents the host response to bacterial plaque, mediated by the environment in which the response occurs. Periodontitis is both site-specific and episodic in nature and thus biomarker development could prove

invaluable in identifying sites with active disease, predicting sites that may develop disease, monitoring response to therapy or identifying patients with enhanced disease susceptibility.

In periodontal disease gingival crevicular fluid (GCF) flows from the gingival microcirculation into the periodontal pockets and the volume increases in proportion to the severity of the local inflammatory process. The study of GCF samples, from defined sites of chronic periodontal inflammation, allows non-invasive access to an inflammatory exudate that could be used for biomarker discovery. GCF contains proteins synthesised and secreted in the inflamed gingival tissues and carried by the GCF to the gingival crevice/pocket. Here, they are augmented by proteins released from bacteria and host cells, particularly polymorphonuclear leukocytes (PMNs), present in the periodontal pocket. The constituents of GCF are therefore derived from a number of sources including microbial plaque, host inflammatory cells, serum and tissue breakdown products. Saliva has also been studied in the search for biomarkers of periodontal disease. Saliva is a more complex fluid, comprising glandular secretions, components of GCF, components of serum and also particles (including bacteria) from a variety of oral and airway sources. Although saliva has the advantage of being easily collected, its biochemical complexity may hinder detection of biomarkers specific for periodontal disease. Furthermore the fact that saliva bathes the whole mouth negates the use of salivary biomarkers for site-specific identification or monitoring of periodontal disease.

Despite an impressive list of possibilities, biomarkers have yet to reach routine clinical use as reasonable predictors of periodontal status. This chapter reviews the analysis of GCF and saliva for monitoring periodontal health and disease. Potentially important biomarkers of disease in both GCF and saliva are highlighted and their merits are described in further detail. Putative biomarkers from both host and bacterial sources are considered and the use of multiple biomarkers is discussed. Following the technological revolution in both genomic and proteomic analysis over the last decade it is tempting to speculate that the next decade could bring much waited progress in the field of biomarker identification and application in the field of periodontal disease.

Chapter V - Periodontal disease represents today the main cause of teeth loss after the third decade of life. About 60% of dental extractions are due to etiopathogenetic periodontal factors. After 35 years, the frequency of marginal periodontal disease varies from 80% to 100% of world population, depending on statistical method used and the demographic areas considered, showing a similar frequency in both sexes, slightly higher in female.

Two important and interrelated factors are involved in its physiopathological progression: 1) the activation of immune system and the release of inflammatory mediators, such as IL-1β, IL-6 and TNF-α, which could overflow into the blood system and induce a systemic inflammatory response; 2) the production of oxygen radicals and their related metabolites.

A recent focus of the dental research is the individuation of biomarkers, which can be easily used as diagnostic tools. Among them, metalloproteinases (MMPs) and heat shock proteins (HSPs) could provide potential biomarkers, which could be useful for evaluating both the periodontitis development and the incidence of the related cardiovascular diseases. Recent studies, in fact, have shown a direct correlation between periodontal and cardiovascular diseases: in particular, both diseases have systemic and local causes, and the constant bacterial contamination of oral cavity could be linked not only to periodontopathy but also to the development of cardiovascular diseases.

To date, the periodontal disease therapy available is based on the individuation and the elimination of the causing factors. Nevertheless, new innovative surgical and pharmacological therapies could be developed.

The aim of this work is to review the literature data focusing on the role of inflammatory mediators and oxidative stress in periodontal disease and related factors.

Chapter VI - Periodontal disease results from complex interactions between infectious agents and host factors. The disease expression can be modified by environmental, acquired, and genetic risk factors. Tobacco usage, especially smoking, is considered a major modifiable risk factor for periodontal disease. In addition to periodontal disease, tobacco usage is also a risk factor for oral cancer and its recurrence, dental cariesand congenital defects in children from mothers who smoke while pregnant. In periodontal disease, smokers have deeper probing depths, more gingival recession, more alveolar loss and more furcation involvement than non-smokers. They also show less favorable responses to various kinds of periodontal treatments including non-surgical, surgical, regenerative procedures and dental implants. It is clear from epidemiology studies that tobacco usage is correlated with periodontal disease. This chapter reviews the evidence for the association between periodontal disease and tobacco, and describes what is currently known about how tobacco and its components affect the periodontal tissues that result in tissue damage.

Chapter VII - A topical issue in periodontology is to find objective diagnostic methods which may be combined with the classical clinical inspection parameters to yield a reliable grading of the severity and extent of periodontal disease. This study deals with a novel cytodiagnostic fluorescence test, performed on exfoliation samples taken from periodontal/oral tissues, useful to assess the severity of periodontal disease. Twenty-one patients with different degrees of periodontitis were subjected to clinical and histopathological grading and the results compared with those obtained from the cytodiagnostic fluorescence assay. The author found that the amount of blood cells (polymorphonuclear and mononuclear leukocytes, erythrocytes), the occurrence of morphologically abnormal epithelial cells, and the number of spirochetes showed a statistically significant correlation with the clinical and histopathological diagnostic parameters, the latter being considered as the most reliable predictors of the severity of periodontal disease. On these grounds, the author suggest that this cytodiagnostic method may greatly help dental practitioners to achieve a chair-side, reliable and objective evaluation of the degree and activity of periodontitis at first dental visit, and to perform a targeted treatment and an accurate follow up of the patients during supportive periodontal therapy.

Chapter VIII -Differentiation of health from disease is central to understanding diagnosis and treatment of periodontal diseases. It is logical to begin with an in-depth examination of the structure and physiology of the healthy periodontium.

Chapter IX - Objectives: The purpose of this study was to compare the short-term clinical effects of a single intrasulcular injection of 2% chlorhexidine gluconate gel (CG) and placebo gel (PG) in orthodontic patients with fixed appliances and established gingivitis aged from 12 to 20 years.

Methods and Materials: 50 patients (31 females, 19 males) were divided into two groups (CG and PG) of 50 subjects. This study was single blind randomized split mouth clinical trial. As randomly assigned by coin toss, the first permanent molars on the right or left side of the mouth received either CG or PG. Probing depth (PD) was measured with a Michigan 0 probe. The gingival index (GI) of Löe and SILNESS and papilla bleeding index (PBI) of

MÜHLEMANN were recorded on the first permanent molars. These indices were measured at baseline, and in treatment on second, fourth, eighth, and the twelfth weeks. T-test and chi-square test were used to analyze the data.

Results: T-test showed that PD was reduced in experimental group in comparison with the control group in the 4th week and following intervals ($p<0.001$). Chi-square showed that PBI was improved in experimental group in comparison with the control group in the 2nd week and following intervals ($p<0.001$). The same test showed that GI was improved in experimental group in the 2nd week and following intervals ($p<0.001$).

Conclusion: The data indicate that the use of a single application of 2% CG was effective in reducing gingivitis related to banded first permanent premolars in adolescents undergoing orthodontic treatment in short time.

Chapter X - The focal infection theory, which for almost half a century justified indiscriminate extraction of teeth to cure focal infections, since the end of the 1940s has become progressively a discarded concept. In parallel with the declining importance assigned to pulp and periapical infections in the pathogenesis of focal diseases, over the last decade there has been increasing interest in the possible relationship between periodontal infection and systemic diseases. Periodontal pathogens and their products, as well as inflammatory mediators produced in gingival tissue, might enter the bloodstream through ulcerated pocket epithelium, causing systemic effects (focal diseases).

On the basis of this mechanism, chronic periodontitis has been implicated as risk factor for cardiovascular diseases associated to atherosclerosis, bacterial endocarditis, diabetes mellitus, respiratory disease preterm delivery, rheumatoid arthritis, and more recently osteoporosis, pancreatic cancer, metabolic syndrome, renal diseases and neurodegenerative diseases such as Alzheimer's disease. Numerous hypotheses, including common susceptibility, systemic inflammation, direct bacterial infection and cross-reactivity, or molecular mimicry, between bacterial antigens and self-antigens, have been postulated to explain these relationships. In this context, the association of periodontal disease with systemic diseases has introduced the concept of "periodontal medicine", which ultimately guides the medical community in therapeutic approaches to improve not only the patient oral health but also systemic health.

This chapter summarizes the pathophysiology of periodontal disease and presentsan update on interrelationships and interactions between periodontal disease and systemic diseases. Moreover, this chapter reviews the published literature that describes the effects of periodontal treatment on cardiovascular diseases, adverse pregnancy outcomes, diabetes mellitus, and respiratory disease.

Chapter XI - Obesity, diabetes and oral diseases (dental cariesand periodontal diseases), largely preventable chronic diseases, are described as global pandemic due their distribution and severe consequences. WHO has called for a global action for prevention and promotion of these diseases as a vital investment in urgent need.

Diabetes and obesity, showing an increasing trend, lead to disabilities and negatively impacts on the quality of life through life course along with oral diseases. WHO projects that the prevalence of diabetes and deaths/year attrituble to diabetes complications will double worldwide by 2030. Globally, more than 1 billion adults are overweight; almost 300 million of them are clinically obese. Being obese/overweight raises steeply the likelihood of developing DM2. Approximately 85% of people with diabetes are DM2, and of these 90% are obese or overweight. Obesity increases the likelihood of periodontitis which is one of the

most common chronic diseases worldwide, described as pandemic, and closely related to DM2. Promoting good oral health is significantly essential for prevention and reducing the negative consequences of periodontal diseases, DM2 and obesity, and to maintain good health, as proposed by European health goals by WHO.

Chapter XII - Periodontitis is one of the predominant polymicrobial infections of humans. Since periodontitis results from complex interactions of multiple microorganisms, it is important to investigate interactions between different periodontal bacteria and host cells. Porphyromonas gingivalis, a gram-negative anaerobe, is a major colonizer of gingival tissues and has been etiologically implicated in periodontal as well as cardiovascular diseases. Cellular invasion by periodontal pathogens including P. gingivalis has been proposed as a possible virulence factor, affording protection from the host immune responses and contributing to tissue damage. In recent periodontal research, polymicrobial infection models have been used to study host response profiles. However, data on the potential of host cell invasion by periodontal pathogens in polymicrobial infection are scarce. The author investigated the ability of periodontal pathogens to modulate invasion of human gingival epithelial cells and aortic endothelial cells by P. gingivalis. Among the pathogens, Fusobacterium nucleatum was shown to significantly enhance the P. gingivalis invasion. The author describe the complex interaction between periodontopathogens and host cells, with a particular focus on the co-infection by P. gingivalis and F. nucleatum.

Chapter XIII -Periodontitis is a major chronic inflammatory disease that destroys periodontal tissue and eventually results in tooth loss. Although periodontitis is a local disease, its chronic status triggers systemic inflammatory diseases including severe type 2 diabetes, heart disease, cancer and atherosclerosis. Therefore, the development of new treatments for periodontitis contributes to the effective inhibition of systemic inflammatory diseases.

High Mobility Group Box-1 (HMGB1), a primarily nuclear protein, is present in many eukaryotic cells and is highly conserved between species. HMGB1 appears to have distinct functions in cellular systems. It acts as an intracellular regulator of transcription and plays a crucial role in the maintenance of DNA function. Extracellular HMGB1 released by various cell types (i.e. macrophages/monocytes, endothelial cells and pituicytes), or necrotic cells, stimulated by lipopolysaccharide (LPS) or tumor necrosis factor-□TNF-□ acts as a proinflammatory cytokine through the multi-ligand receptor for advanced glycation end-products (RAGE) and toll-like receptors (TLRs) 2 and 4. Extracellular HMGB1 plays a critical role in the progression of chronic inflammatory diseases, such as septic shock, rheumatoid arthritis, diabetes and atherosclerotic lesions. Recent studies show that HMGB1 is continuously released from gingival epithelial cells modulated by TNF-□ and expressed in epithelial tissues of patients with periodontitis. HMGB1 may be involved in the progression of periodontitis as a novel inflammatory mediator. Therefore, understanding the mechanisms underlying the functions of HMGB1 may lead to novel therapeutic approaches for chronic periodontitis and help to prevent systemic inflammatory diseases.

This review summarizes the current knowledge on HMGB1, including its correlation with disease and preventive medicine.

Chapter XIV - Chronic periodontal diseases include a group of inflammatory diseases that affect periodontal supporting tissues of the teeth and encompass destructive and nondestructive conditions. Periodontal diseases are multifactorial and the role of dental biofilm in their initiation is primary. However, whether dental biofilm affects a particular

subject, what form the disease takes and how it progresses, are all dependent of a wide variety of factors. Therefore, the objective of this chapter is to outline the risk factors described for the most prevalent chronic periodontal diseases (plaque induced gingivitis and chronic periodontitis) and to explain some basic concepts related to the current understanding of the role of these risk factors based on in vitro, animal and human studies. The review will focus on the factors that may be associated with a direct increase in the likelihood of occurrence of disease or an increase in its severity. The following factors will be discussed: 1) host characteristics, such as age, gender and race; 2) social and behavioral factors (socioeconomic status, cigarette smoking and emotional stress); 3) systemic factors, e.g. diabetes mellitus and osteoporosis; 4) genetic factors; 5) tooth-level factors (root grooves, tooth position, caries, occlusal discrepancies, iatrogenic restorations, root abnormalities and periodontal parameters); and 6) the microbial composition of dental biofilm. Finally, this chapter will also present literature-based evidence on predictive factors associated with patients and tooth susceptibility for recurrence of periodontitis after the end of the active periodontal therapy and will examine the use of some prognostic models which may be useful for clinicians in the identification high-risk groups of patients.

Chapter XV - The oral cavity is a warm, moist environment, in which a number of microorganisms colonize and live in harmony as a community, a so-called biofilm. In this environment, antimicrobial peptides may play a critical role in maintaining normal oral health and controlling innate and acquired immune systems in response to continuous microbial challenges in periodontal disease. Two major families of antimicrobial peptides, found in the oral cavity, are defensin and cathelicidin. Members of the defensin family are cysteine-rich peptides, synthesized by plants, insects, and mammals. These peptides vary in length and in the number of disulfide bonds, and have a beta-sheet structure. In the oral cavity, four alpha-defensins are synthesized and stored in neutrophil granules, which are converted into active peptides by proteolytic processing, while three human beta-defensins (hBDs), hBD-1, hBD-2, and hBD-3, are predominantly produced by oral epithelial cells. The only member of the cathelicidin family found in humans is LL-37, an alpha-helical peptide that contains 37 amino acids and begins with two leucines at its NH3-terminus. LL-37 is derived from enzymatic cleavage of a precursor peptide, namely, human cationic antimicrobial peptide-18. Clinically, differential expression of antimicrobial peptides has been reported in specific types of periodontal disease, and their presence has been shown in saliva and gingival crevicular fluid. Current evidence suggests that alpha-defensins, beta-defensins, and LL-37 have distinct, but overlapping, roles in antimicrobial and pro-inflammatory activities. Several studies have shown antimicrobial activities of hBD-2, hBD-3, and LL-37 against several periodontal pathogens, suggesting their potential role as antimicrobial agents for periodontal disease. The clinical significance of antimicrobial peptides in periodontal disease has recently been demonstrated in morbus Kostmann syndrome, a severe congenital neutropenia, in which chronic periodontal infection in young patients, resulting from a deficiency of neutrophil-derived antimicrobial peptides, causes early tooth loss. Although researchers initially focused their attention on antimicrobial activities, it is now becoming evident that defensins and LL-37 are multifunctional molecules that mediate various host immune responses, and may thus represent essential molecules of innate immunity in periodontal disease. In this chapter, basic knowledge and the clinical importance of antimicrobial peptides in periodontal disease will be discussed in detail.

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Chapter I

Aesthetic Periodontal Therapy – Root Coverage

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Abstract

Aesthetic considerations have influenced the management of dental maladies in varying degrees for many years. For many years the goals of periodontal surgery have been determined by functional aspects only. During recent years periodontal surgery has shifted its focus from achieving more functional goals toward a combination of both good functional and esthetic results. While accomplishing the best possible functional result, esthetics should not only be maintained, but also enhanced. Sometimes the esthetic outcome is the only important factor and function becomes secondary (e.g. treatment of recessions or the creation of papillae). Predictability becomes the key word in this type of periodontal surgery. Patient awareness and expectations have increased recently to the point that less than optimal esthetics is no longer an acceptable outcome. Periodontal plastic surgery would accordingly be defined as "surgical procedures performed to prevent or correct anatomic, developmental, traumatic or disease induced defects in the gingiva, alveolar mucosa or bone".The present chapter is presenting and discussing the clinical outcomes of several root coverage techniques: pedicle soft tissue grafts, rotational flaps, coronally advanced flap, semilunar flap, free soft tissue graft, nonsubmerged grafts, submerged grafts etc.

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1. Introduction

Aesthetic considerations have influenced the management of dental maladies in varying degrees for many years. For many years the goals of periodontal surgery have been determined by functional aspects only. During recent years periodontal surgery has shifted its focus from achieving more functional goals toward a combination of both good functional and esthetic results. While accomplishing the best possible functional result, esthetics should not only be maintained, but also enhanced. Sometimes the esthetic outcome is the only important factor and function becomes secondary (e.g. treatment of recessions or the creation of papillae). Predictability becomes the key word in this type of periodontal surgery (Hurzeler and Weng, 1999).

Mucogingival surgery is a broaderterm that includes nonsurgical procedures such as papilla reconstruction by means of orthodontic or restorative therapy (Takei et al., 2006).*Periodontal plastic surgery* is defined as "surgical procedures performed to prevent or correct anatomic, developmental, traumatic or disease induced defects in the gingiva, alveolar mucosa or bone". Among treatment procedures that may fall within this definition are various soft and hard tissue procedures aiming at: gingival augmentation, root coverage, correction of mucosal defects at implants, augmentation of edentulous ridges, removal of aberrant frenulum, prevention of bridge collapse associated with tooth extraction, crown lengthening, mucogingival tattoo, open interproximal space, gingival enlargement and exposure of teeth that are not likely to erupt (Wennström and Pini Prato, 1997; McGuire, 1998).

The present chapter is presenting and discussing the clinical outcomes of several root coverage techniques.

2. Gingival Recession

Gingival recession is characterized by the displacement of the gingival margin apically from the cementoenamel junction, or CEJ, or from the former location of the CEJ in which restorations have distorted the location or appearance of the CEJ. Gingival recession can be localized or generalized and be associated with one or more surfaces. The resulting root exposure is not esthetically pleasing and may lead to sensitivity and root caries(Kassab and Cohen, 2003).

In USA, it was revealed that the prevalence of ≥1 mm recession in persons 30 years and older was 58%, representing 61.3 million adults, and the extent of ≥1 mm recession averaged 22.3% teeth per person. The extent of ≥1 mm recession was 38.4% teeth per person among persons with gingival recession. The prevalence and extent of recession increased steadily with the age of the cohort, regardless of the threshold level used in defining recession. In the youngest age cohort (30 to 39 years), the prevalence of recession was 37.8% and the extent averaged 8.6% teeth. In contrast, the oldest cohort, aged 80 to 90 years, had a prevalence of 90.4% (more than twice as high), and the extent averaged 56.3% teeth (more than six times as large). A comparisonby gender and race/ethnicity showed that the prevalence and extent of recession were significantly higher in males than females ($P< 0.001$) after adjusting for age and race/ethnicity, and in blacks than in whites ($P< 0.002$), after adjusting for age and gender (Albandar and Kingman, 1999).

Several factors were related to the etiology of gingival recession (Kassab and Cohen, 2003):

- Aging
- Anatomical factors that have been related to recession include fenestration and dehiscence of the alveolar bone, abnormal tooth position in the arch, aberrant path of eruption of the tooth, individual tooth shapeand presence/lack of attached gingiva
- Physiological factors may include the orthodontic movement of teeth to positions outside the labial or lingual alveolar plate, leading to dehiscence formation.
- Various forms of trauma—such as vigorous toothbrushing, aberrant frenal attachment, occlusal injury, operative procedures and tobacco chewing—have been thought to play a role in the etiology of recession.

According to Miller (1985), recession defects can be classified into four groups taking into consideration the anticipated root coverage that can be obtained:

- Class I: Marginal tissue recession not extending to the mucogingival junction. No loss of interdental bone or soft tissue.
- Class II: Marginal tissue recession extends to or beyond the mucogingival junction. No loss of interdental bone or soft tissue.
- Class III: Marginal tissue recession extends to or beyond the mucogingival junction. Loss of interdental bone. Interdental soft tissue is apical to the cemento-enamel junction, but coronal to the apical extent of the marginal tissue recession.
- Class IV: Marginal tissue recession extends beyond the mucogingival junction. Loss of interdental bone and to a level corresponding to the apical extent of the marginal tissue recession.

While complete root coverage can be achieved in Class I and II type recession defects, only partial coverage may be expected in recessions of Class III and IV.

However, this classification has some limitations (Bouchard et al., 2001):

- The position of the tooth and the alveolar ridge are not taken into account. Recessions in teeth in a labial position may require orthodontic treatment prior to surgical procedures.
- The size of the defect in both vertical and horizontal dimensions must be considered. As a rule of thumb, the literature classifies the defects as shallow (<3 mm), moderate (3 to 5 mm) or deep (>5 mm). On average, clinical studies indicate a defect width of 4.5 mm. A 5-mm width should be viewed as wide. It is to be assumed that the larger the recession area, the less root coverage should be expected.
- The residual depth of the vestibule also seems to be of importance for the selection of procedures.

A new two-figure Index of Recession (IR) was described by Smith (1997). The *horizontal* component - *the first digit* - is expressed as a whole number value from the range 0-5 depending on what proportion of the CEJ is exposed, on either the facial or lingual

aspects of the tooth, between the mesial and distal midpoints (MM-MD distance) approximally. The criteria are as follows:

- 0; no clinical evidence of root exposure
- 1: as 0, but a subjective awareness of dentinal hypersensitivity in response to a 1 second air blast is reported and/or there is clinically detectable exposure of the CEJ for up to 10% of the estimated MM-MD distance: a slit like defect
- 2; horizontal exposure of the CEJ >I0% but not exceeding 25% of the estimated MM-MD distance
- 3: exposure of the CEJ >25% of the MM-MD distance but not exceeding 50%
- 4; exposure of the CEJ >50% of the MM-MD distance but not exceeding 75%
- 5: exposure of the CEJ >75'/o of the MM-MD distance up to 100%

Allocation of these codes does not imply that the extent of recession is equally dispersed about the facial or lingual midpoints of the area of exposed roots.

The second digit of the IR gives the vertical extent of recession measured in whole mm on a range 0-9. The precise criteria proposed are as follows:

0: no clinical evidence of root exposure

1: as 0, but a subjective awareness of dentinal hypersensitivity is reported and/or there is clinically detectable exposure of the CEJ not extending >1 mm vertically to the gingival margin

2-8: root exposure 2-8 mm extending vertically from the CEJ to the base of the soft tissue defect

9: root exposure>8 mm from the CEJ to the base of the soft tissue defect.

An asterisk is afixed to the second digit whenever the vertical component of the soft tissue defect encroaches into the muco-gingival junction or extends beyond it into alveolar mucosa. The absence of an asterisk thus implies either absence of muco-gingival junction at the indexed site or its non-involvement in the soft tissue defect. The prefixed F (or L) denotes whether gingival recession is facial {or lingual) to the involved root.

3. Gingival Biotypes

The gingival morphology of the maxillary anterior region plays an important role in determining the final esthetic outcome (Fu et al., 2010).

Clinical observations have led clinicians to identify two basic human periodontal forms (Ochsenbein and Ross, 1973; Claffey and Shanley, 1986; Seibert and Lindhe, 1989). The more prevalent, the thick flat type, occurs in over 85% of the patient population; the other, the thin scalloped type, occurs in less than 15% of cases (Sanavi et al., 1998).

In the thick flat type there is this normal rise and fall of the gingival and bone, but there is not a great disparity between the direct facial and that found interproximally. The gingiva is thick or dense and is fibrotic in nature. Usually this type of periodontiurn has, quantitatively and qualitatively, adequate amounts of attached masticatory mucosa. The teeth found in the

thick flat periodontium are usually characterized by being more bulbous and square in form. Contact areas are located more apically and usually are broad inciso gingivally and faciolingually. The interproximal papillae filling the space between the teeth terminate at the contact areas, hence, a flat periodontium. When irritated by tooth preparation, impression procedures, extraction, or other clinical techniques, this periodontium usually reacts with inflammation, followed by migration of the junctional epithelium apically, with resultant periodontal pocket formation or redundant tissue (Sanavi et al., 1998). Predicable soft and hard tissue contour after healing following surgery and minimal ridge resorption occurs after extractions (Kao et al., 2008).

The thin scalloped type of periodontium, on the other hand, is distinguished by a pronounced disparity between the height on the direct facial and that found interproximally. The underlying bone is usually thin on the facial with dehiscences and fenestrations commonly found. Usually there is less attached masticatory mucosa, from both quantitative and qualitative perspectives. In the thin scalloped periodontium, the tooth form is usually more subtle and somewhat triangular. Contact areas are located more incisally and are small incisogingivally and faciolingually. The cervical convexity is less prominent. Since the contact areas are located more incisally, the interproximal papilla is also positioned more incisally, hence, the scalloped form. Excessive irritation of this type of periodontium usually leads to recession both facially and interproximally (Sanavi et al., 1998). In this gingival biotype after surgery it is difficult to predict where tissue will heal and stabilize and extensive ridge resorption in the apical and lingual direction usually occurs after extractions (Kao et al., 2008).

Many methods have been proposed to measure gingival tissue thickness:

- direct measurements (Greenberg et al., 1976)
- probe transparency (DeRouck et al., 2009; Kan et al., 2003). This evaluation was based on the transparancy of the periodontal probe through the gingival margin while probing the sulcus at the midfacial aspect of the examined tooth. If the outline of the underlying periodontal probe could be seen through the gingival, it was categorized as thin; if not, it was categorized as thick.
- ultrasonic devices (Müller et al., 2000)
- cone-beam computer tomography (CBCT) (Januário et al., 2008; Barriviera et al., 2009; Fu et al., 2010).

The identification of the gingival biotype may be important in clinical practice since differences in gingival and osseous architecture have been shown to exhibit a significant impact on the outcomes of periodontal therapy (Claffey and Shanley., 1986; Anderegg et al., 1995; Baldi et al., 1999), root coverage procedures (Huang et al., 2005; Hwang and Wang, 2006), orthodontic therapy (Wennström et al., 1990, 1996) and implants esthetics (Zigdon et al., 2008; De Rouck et al., 2009; Evans and Chen, 2008; Romeo et al., 2008).

Hwang and Wang (2006) reviewed the current literature to verify the presence of any association between gingival thickness and root coverage outcomes.Fifteen investigations were included. All of these reported at least 0.7mm of flap thickness, although measurement locations varied. Treatment modalities included coronally advanced flap, connective tissue graft, and guided tissue regeneration with and without adjuncts. A significant moderate

correlation occurred between weighted flap thickness and weighted mean root coverage and weighted complete root coverage (r = 0.646 and 0.454, respectively; weighted mean of gingival thickness accounted for 41.7% of variability in weighted mean root coverage results and a lesser proportion (20.7%) in weighted complete root coverage (Hwang and Wang, 2006).

The paradigm shift proposedby Kao et al. (2008) was that by taking into consideration the gingival tissue biotype during treatment planning, more appropriate strategies for periodontal management may be developed, resulting in more predictable treatment outcomes.

4. Root Coverage Procedures

Surgical procedures used in the treatment of recession defects may basically be classified as (1) *pedicle soft tissue graft procedures* and (2) *free soft tissue graft procedures* (Wennström et al., 2008).

4.1. Pedicle Soft Tissue Grafts

The pedicle graft procedures are, depending on the direct of transfer, grouped as (1) rotational flap procedures (e.g. laterally sliding flap, double papilla flap, oblique rotated flap) or (2) advanced flap procedures (e.g. coronally repositioned flap, semilunar coronally positioned flap). Regenerative procedures are also included within the group of pedicle graft procedures, i.e. rotational and advanced flap procedures involving the placement of a barrier membrane between the graft and the root or the application of enamel matrix proteins (Wennström et al., 2008).

4.1.1. Rotational Flaps

Grupe and Warren (1956) introduced the first technique for covering a localized gingival recession. The laterally sliding flap consists of the removal of the collar of the gingiva around the area of recession and elevation of a full-thickness flap on the adjacent tooth.This flap is positioned laterally and sutured over the denuded root surface. The limitations of the procedure are the amount of the attached tissue and the thickness of the labial bone at the donor site. Leaving a thin labial plate exposed on the donor tooth risks recession at this site. An laterally positioned pedicle graft cannot be performed unless there is significant gingival lateral to the site of recession. A shallow vestibule also may jeopardize outcomes. Although the use of the laterally positioned pedicle graft provides an ideal color match, it often is inadequate for the treatment of multiple recessions (Kassab et al., 2010).

The technique is as follows (Figure 1.):

*Recipient area.*Initially, the recipient area for the laterally moved flap is prepared. A reverse bevel incision is made all along the soft tissue margin of the defect. After removal of the dissected pocket epithelium, the exposed root surface is thoroughly curetted. Two superficial incisions are then delineating a 3 mm wide recipient area, at the one side of the

defect as well as apical to the defect, where the epithelium together with the outer portion of the connective tissue is removed by sharp dissection (Wenestrom et al., 2008).

The flap design is outlined by two vertical incisions that extended from the horizontal incision to several millimeters apically to the mucogingival junction. A horizontal incision is performed either at the gingival or 3 mm apically, following the marginal gingival contour, thus joining the vertical incisions. A beveled linear horizontal incision is performed to optimize the content of keratized tissue in the flap when the donor site is an edentulous site. The flap is elevated as full thickness in the portion adjacent to the recession and as partial thickness in the portion distal to the recession. Partial-thickness dissection is continued apically and laterally to obtain passivity of flap movement and absence of muscle pull or periosteal adhesion. The flap is rotated laterally to cover the recession defect completely and extend for approximately 1 mm coronal to the cemento-enamel junction. Careful flap suturing is performed to position and secure the soft tissues over the root surface by means of sling and simple sutures (Santana et al., 2010).

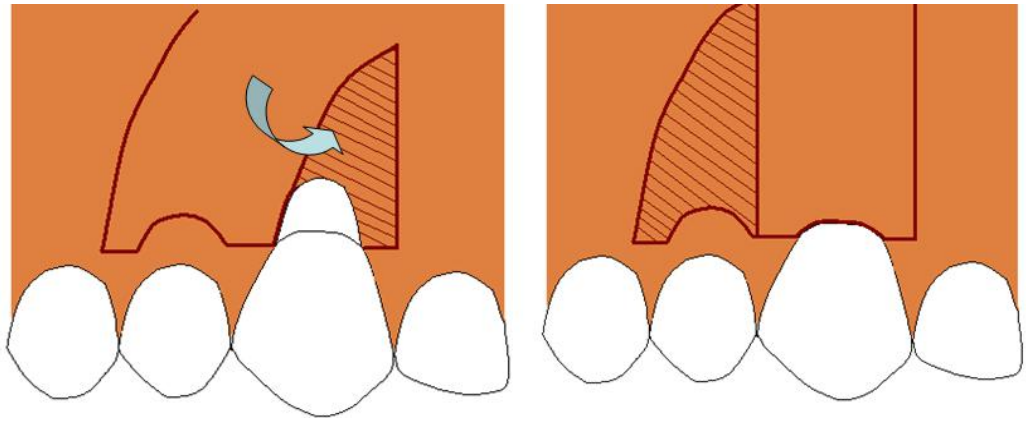

Figure 1. Schematic drawing of rotational flap procedure.

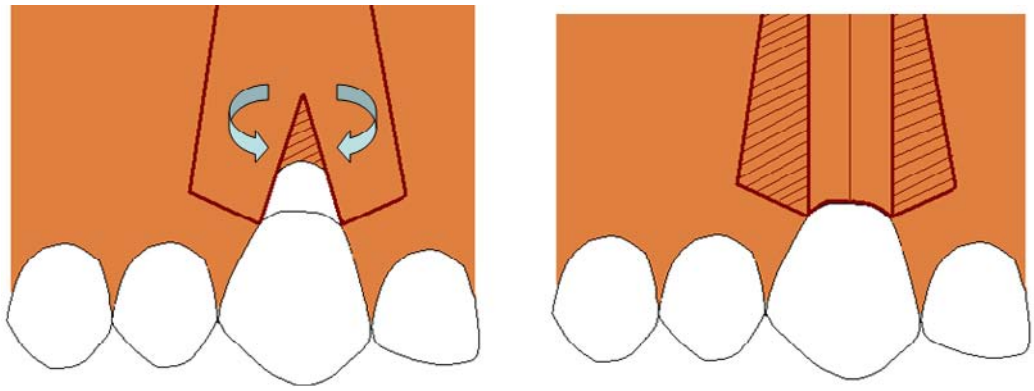

Figure 2. Schematic drawing of double papilla flap technique.

Following removal of the dressing and the sutures, usually after 10-14 days, the patient is instructed to avoid mechanical tooth cleaning for further 2 weeks, but to use twice daily rinsing with chlorhexidine solution as a means of infection control (Wenestrom et al., 2008).

Several modifications have been described to overcome the problem of dehiscence at the donor site. Staffileno (1964) used a split-thickness pedicle flap so as not to denude the adjacent site. This approach compromises vascularity and does not preclude bone resorption at the donor site (Bahat et al., 1990). Other modifications of the procedure are the oblique rotated flap (Pennel et al., 1965), the rotation flap (Patur, 1977), the double papilla flap (Cohen and Ross, 1968) (Figure 2.) and the transpositioned flap (Bahat et al., 1990).

Zucchelli et al. (2010) revealead that present data do not seem to indicate the laterally moved flap is an highly predictable and effective root coverage surgical procedure. From the studies reviewed, the reported mean percentage of root coverage ranges between 34% and 82% (Smuckler,1976; Guinard and Caffesse, 1978; Espinel and Caffesse 1981; Waite, 1984; Zade and Hirani, 1985; Oles et al., 1985) and only Oles et al. (1988) reported data relating the "percentage of complete (up to the cemento-enamel junction) root coverage" and the range was between 40% and 50% (Zucchelli et al., 2010).

4.1.2. Advanced Flaps Procedures

Since the lining mucosa is elastic, a mucosal flap raised beyond the mucogingival junction can be stretched in coronal direction to cover exposed root surfaces. The coronally advanced flap procedure has been described by several authors (Allen and Miller Jr, 1985; Harris and Harris, 1994; Milano, 1998; Romanos et al., 1993; Wennström and Zucchelli, 1997; Bernimoulin et al., 1975).

The coronally advanced flap is the first choice surgical technique when there is adequate keratinized tissue apical to the recession defect. Optimum root coverage results, good color blending of the treated area with respect to adjacent soft tissues, and recuperation of the original morphology of the soft tissues margin can be predictably accomplished using this surgical approach. Furthermore, the coronally advanced flap is very effective in treating multiple recession defects affecting adjacent teeth with obvious advantages for the patient in terms of esthetics and morbidity. Some unfavorable local anatomic conditions may render the coronally advanced flap contraindicated: 1) the absence of keratinized tissue apical to the recession defect; 2) the presence of gingival ("Stillman") cleft extending in alveolar mucosa; 3) the marginal insertion of frenuli; 4) the presence of deep root structure loss; or 5) presence of a very shallow vestibulum. In these situations the clinician should take the soft tissues located laterally to the recession defect into consideration to evaluate the possibility to perform a laterally moved flap (Zucchelli et al., 2010; Wennström and Zucchelli, 1996; Zucchelli and De Sanctis, 2000).

The coronally positioned pedicle graft has many advantages over other surgical procedures used to cover exposed roots. It does not require a separate surgical site to obtain a graft. The tissue utilized will be a perfect color and contour match with the surrounding tissue. Additionally, the procedure is simple to perform and does not require a lot of time (Harris and Harris, 1994).

In aim to evaluate the predictability of the procedure several clinical studies have been evaluated by Bouchard et al., 2001. The mean depth of the recession defects treated was 3.7 mm (3.3–4.1mm). The mean % of root coverage for advanced flaps was reported to be 77%

(55–98), while the % of teeth with complete root coverage was 45% (9-84%) (Bouchard et al., 2001).

More recently, Cairo et al. (2008) reviewed the clinical outcomes of the coronally advanced flap on a total of 794 Miller Class I and II gingival recessions in 530 patients from 25 RCTs. This systematic review confirms that the coronally advanced flap procedure is a safe and reliable approach in periodontal plastic surgery and is associated with consistent recession reduction and frequently with complete root coverage. The results of meta-analyses showed that only two combinations (coronally advanced flap + connective tissue graft and coronally advanced flap + enamel matrix derivative) provided better results than coronally advanced flap alone. Coronally advanced flap + connective tissue graft resulted in better clinical outcomes for both complete root coverage (OR=2.49) and recession reduction (10.49 mm) compared with coronally advanced flap, and no other therapy provided better results than coronally advanced flap + connective tissue graft. The combination of coronally advanced flap + enamel matrix derivative was associated with a higher probability to obtain complete root coverage (OR=3.89) and a higher amount of recession reduction (0.58 mm) than coronally advanced flap alone. A possible benefit following root coverage procedures may be the augmentation of keratinized tissue. This systematic review showed that coronally advanced flap + connective tissue graft was associated with better clinical outcomes in terms of keratinized tissue gain following therapy.

The technique for the coronally advanced flap procedure is:

The coronally advanced flap is initiated by two horizontal bevelled incisions (3mm in length), mesial and distal to the recession defect located at a distance from the tip of the anatomical papillae equal to the depth of the recession plus 1 mm.Two bevelled oblique, slightly divergent, incisions starting at the end of the two horizontal incisions and extending to the alveolar mucosa. The resulting trapezoidal-shaped flap is elevated with a split–full–split approach in the coronal–apical direction. In order to permit the coronal advancement of the flap, all muscle insertions present in the thickness of the flap are eliminated. This is done keeping the blade parallel to the external mucosal surface. Coronal mobilization of the flap is considered “adequate” when the marginal portion of the flap was able to passively reach a level coronal to the CEJ of the tooth with the recession defect. In fact, the flap should be stable in its final coronal position even without the sutures. The root surface is mechanically treated with the use of curettes. It must be considered that only the portion of the root exposure with loss of clinical attachment (gingival recession1 probeable gingival sulcus/pocket) is instrumented. Exposed root surfaces belonging to the area of anatomic bone dehiscence were not instrumented not to damage connective tissue fibres still inserted in to the root cementum. The facial soft tissue of the anatomic inter-dental papillae coronal to the horizontal incisions is disepithelized to create connective tissue beds to which the surgical papillae of the coronally advanced flap are sutured. By moving the flap coronally to reach the tip of the disepitelized anatomical papillae, the vestibular soft tissue should be positioned 1 mm coronal to the cemento-enamel junction to account for soft tissue shrinkage. The suture of the flap is started with two interrupted periosteal sutures performed at the most apical extension of the vertical releasing incisions; then, it proceeded coronally with other interrupted sutures, each of them directed, from the flap to the adjacent buccal soft tissue, in the apical–coronal direction. This is done to facilitate the coronal displacement of the flap and to reduce the tension on the last coronal sling suture (De Sanctis and Zucchelli, 2007) (Figure 3.).

Figure 3. Coronally advaced flap procedure. a. A recession defect on the lower canine. b. Close suturing of the pedicle graft to cover the exposed root surface. c. Healing outcome 3 months post-operatively. d. Healing outcome 1 year post-operatively.

For the treatment of isolated gingival recession, Zucchelli et al. (2004) proposed the use of a *laterally moved and coronally advanced flap*. Thereafter, the proposed surgical technique combined the root coverage and esthetic advantages of the coronally advanced flap with the increase in gingival thickness and in the amount of keratinized tissue associated with the use of the laterally moved flap and resulted in a very high mean percentage of root coverage (96%) and complete soft tissue root coverage (up to the CEJ) accomplished in 80% of treated cases.

The main modification of the present surgical technique, with respect to those previously proposed, was the elimination of all muscle insertions in the thickness of the flap to permit the coronal advancement of the laterally moved flap. Furthermore, the coronal advancement of the flap allowed the surgical papillae to cover the anatomic papillae which represented the most coronal areas for anchoring the flap and a critical source for vascular exchanges. In addition, coronal advancement of the flap beyond the cemento-enamel junction likely compensates for the post-surgical soft tissue contraction, resulting in no exposure of the root surface (Zucchelli et al., 2004).

The different thickness during flap elevation (greater in the central area than in the more peripherical portions of the flap) represented another aspect of the proposed surgical technique. In a thicker flap the amount of vascularized connective tissue increases and the post-surgical soft tissue contraction decreases. Both these factors improve the possibility of accomplishing and maintaining root coverage (Zucchelli et al., 2004).

Another feature of the present surgical technique was the sequence of sutures: the apical stabilization sutures in the most apical extension and along the releasing incision and the double mattress horizontal suture at the fornix were performed before the marginal sling suture. Thus the most marginal portion of the flap was stable in the desired coronal position without disrupting forces acting on it at the time of the final suture. Furthermore the double mattress suture reduced lip tension on the marginal portion of the flap during the first healing period and avoided the use of surgical periodontal dressing (Zucchelli et al., 2004).

The Semilunar Flap

The coronally positioned flap may be preferable in situations with multiple recession defects. In situations with only shallow defect the semilunar coronally positioned flap described by Tarnow (1986) offers an alternative approach.

The semilunar coronally repositioned flap has the following advantages: (1) there is no tension on the flap after coronally repositioning it; (2) there is no shortening of the vestibule; (3) the papillae mesial and distal to the toothbeing treated remain cosmetically unchanged; (4) no sutures are needed because the lack of tension of the tissue being coronally positioned (Tarnow, 1986).

The technique for the semilunar coronally repositioned is:

A semilunar incision is placed apically to the recession and at a distance from the soft tissue margin, which should be approximately 3 mm greater than the depth of the recession (Figure 4.). The outline of the incision should be parallel to the curvature of the gingival margin. The incision is extended into the papilla region on each side of the tooth, but care should be taken to secure a collateral blood supply to the pedicle graft. A split thickness dissection of the facially located tissue is then made by an intracrevicular incision extending apically to the level of the semilunar incision. The mid-facial soft tissue graft is coronally repositioned to the level of the cemento-enamel junction and stabilized by light pressure for 5 min.No suturing is needed but a light curing dressing is applied for wound protection (Wennström and Pini Prato, 1997).

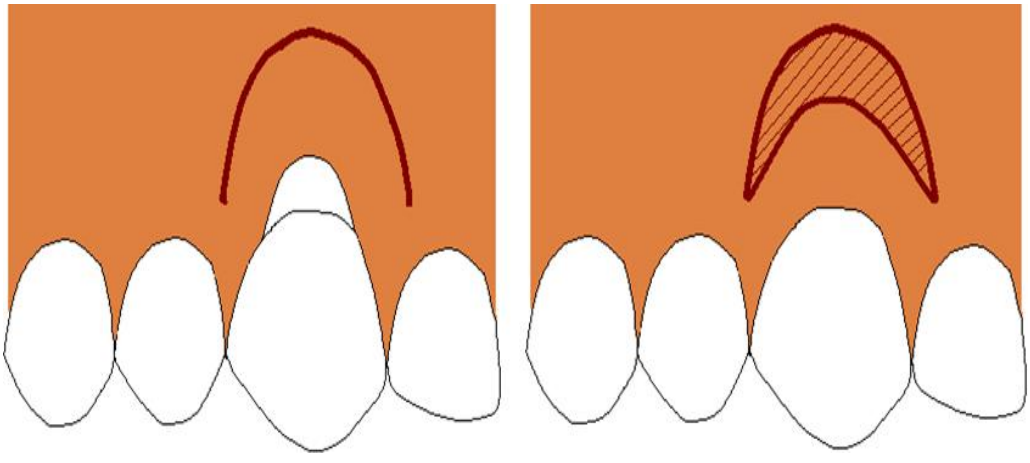

Figure 4. Schematic drawing of semilunar coronally repositioned flap.

Despite the lack of tension in the mobilized pedicle, its stability in the more desired coronal position is questionable, as no suturing of the advanced flap portion is indicated. This is of particular concern when the procedure is considered for teeth with highly scalloped gingival margins, where coronally manipulating the tissue could be more demanding (Haghighat, 2006). The semilunar flap is a modification of a technique described in the late 1960s for incisally repositioning the gingival tissues to address recession defects on labial surfaces of maxillary cuspids (Sumer, 1969; Haghighat, 2006).

In a in a split-mouth design the outcome of gingival recession therapy using subepithelial connective tissue graft or the semilunar coronally positioned flap procedure was evaluated by Bittencourt et al. (2006). No statistically significant differences were observed between groups in any of the clinical parameters at baseline. Recession height, recession width, width of keratinized tissue, thickness of keratinized tissue, probing depth, and clinical attachment level were measured at baseline and 6 months post-surgery. In the subepithelial connective tissue graft group, recession height decreased from 2.20 ± 0.56 mm to 0.21 ± 0.25 mm, corresponding to a mean root coverage of 90.95% ± 11.46%. In the semilunar coronally positioned flap group, recession height decreased from 2.15 ± 0.59 mm to 0.10 ± 0.19 mm, corresponding to a mean root coverage of 96.10% ± 7.69. Complete root coverage was accomplished in 52.94% of the treated cases in the subepithelial connective tissue graft group and in 76.47% in the semilunar coronally positioned flap group (Bittencourt et al., 2006).

After 30 months, the mean percentages of root coverage were 89.25% and 96.83% for the semilunar coronally positioned flap and subepithelial connective tissue graft groups, respectively. Complete root coverage at the final observation was achieved in 58.82% of the treated cases in the semilunar coronally positioned flap group and in 88.24% of the patients in the subepithelial connective tissue graft group. The comparison between 6 and 30 months showed that two patients in the subepithelial connective tissue graft group gained attachment and achieved complete root coverage; this only occurred in one patient in the semilunar coronally positioned flap group. The subepithelial connective tissue graft group maintained a statistically significant increase in thickness of keratinized tissue ($P<0.05$) at 30 months. At this time, there were no significant differences between the two groups with regard to recession height, recession width, width of keratinized tissue, thickness of keratinized tissue, probing depth and clinical attachment level. With regard to esthetic improvement, after 30 months, patients in semilunar coronally positioned flap and subepithelial connective tissue graft groups were generally satisfied with both procedures (82.3% and 100%, respectively). Although they presented similar good results, more patients preferred, based on esthetics achieved, treatment with subepithelial connective tissue graft. This can be explained by the higher percentage of complete RC and the absence of hypertrophic scars or fibrosis in this group, whereas in the semilunar coronally positioned flap group, seven patients complained about the presence of hypertrophic scars, although they were not visible while smiling (Bittencourt et al., 2009).

The modified semilunar coronally advanced flap for the correction of gingival recession present on adjacent teeth was described by Haghighat (2006). Semilunar incisions were made apical to the recession defects, starting within mucosa and extended mesio-distally, arching more coronally to terminate apical to the papillae mesial and distal to the teeth exhibiting the defects. The papilla between the teeth with recession was coronally advanced after a split thickness dissection and sutured more coronally, over the deepithelialized portion of the original papilla.

This technique provides better control over flap repositioning than previously described semilunar coronally advanced flaps and reduces the likelihood of apical tissue retraction when attempting root coverage on two adjacent teeth. This is particularly of value for highly scalloped gingival margins where coronal manipulation and stability are difficult. As described with the original semilunar flap procedure, adequate thickness and width of keratinized tissue apical to the recession defect are required. In cases exhibiting a thin-tissue biotype, tissue augmentation either before or at the time of the corrective surgery is advocated. Therefore, the technique is of value in the correction of residual recession defects on two adjacent teeth where previous attempts at coverage using soft tissue autografts have been made (Haghighat, 2006).

4.1.3. Pedicle Soft Tissue Graft Procedures Combined with a Barrier Membrane

Regeneration is defined as "a reproduction or reconstitution of a lost or injured part. It is, therefore, the biologic process by which the architecture and function of lost tissues are completely restored." This implies regeneration of the tooth's supporting tissues, including alveolar bone, periodontal ligament, and cementum. Many studies have attempted to achieve regeneration, but success rates have varied from minimal or partial regeneration to almost complete regeneration. The use of GTR has been suggested for treatment of recession (Kassab et al., 2010).

In considering healing dynamics of the root-gingiva interface, regeneration may be influenced by factors related to morphological characteristics of the recession defect, the surgical manipulation as well as traumatic events during the early healing phase. The lack of a horizontal and angular component of the associated bone defect facilitates close proximity of the exposed root surface to proliferating cells assumed necessary for regeneration of the site. It is reasonable to assume that the deeper and narrower the defect, the greater the periodontal regeneration occurring away from disturbing environmental factors. Factors such as tooth location, vestibular depth, and muscular and frenum insertions may affect wound stability once a pedicle flap is advanced onto an exposed root. Flap management, suturing technique, and post-surgery wound protection should be adapted to the peculiar anatomic conditions of the gingival recession defect to optimize wound stabilization (Trombelli, 1999).

The last decade has seen an increasing number of clinical reports on guided tissue regeneration (GTR) for reconstruction of gingival recession defects. Danesh-Meyer and Wikesjo (2001) evaluated the efficacy of GTR procedures to provide root coverage in gingival recession defects and reviewed studies and case-series using nonresorbable and bioresorbable membranes, studies comparing GTR to the subepithelial connective tissue graft procedure, and histologic reports of healing following GTR, published in the English language from 1985 to 2000 (Danesh-Meyer and Wikesjo, 2001).

Root coverage among the studies using nonresorbable membranes averaged 3.5±0.7 mm. Clinical attachment level gain averaged 4.0±0.9 mm. Importantly, probing depths in the augmented sites remained shallow following the GTR protocol. Limited mean increase in keratinized gingiva (0.6±0.8 mm) was observed among studies using non-resorbable membranes. Keratinized gingivas ranged from 1.0 to 1.9 mm pre-treatment compared to 0.5 to 6.2 mm post-treatment. A majority of studies reviewed reporting observations of membrane exposure, this compromise of the efficacy of GTR is an important consideration in

the general utility of this technology for gingival recession defects (Danesh-Meyer and Wikesjo, 2001).

To eliminate the need for a second surgical procedure to remove a nonresorbable membrane, the use of various bioabsorbable materials has been proposed (Kassab et al., 2010).

The majority of studies evaluating bioresorbable membranes for treatment of gingival recession defects are case studies typically involving only few subjects. The variety of biomaterials complicates any comparisons between studies as the materials may differ in physical properties including biocompatibility, cell exclusion, clinical manageability, tissue integration, space provision, space maintenance, and bioresorbtion, all of which may inffluence their ultimate relevance as GTR devices. Root coverage among the studies using bioresorbable membranes averaged 2.8±1.2 mm. CAL gain averaged 2.5±1.3 mm. As observed for nonresorbable membranes, probing depths remained shallow following the GTR protocol. Bioresorbable membranes appear less effective than the nonresorbable membrane technology in more limited gingival recession defects, however this relative deficiency appears compensated in advanced defects. As observed for the nonresorbable membrane technology, keratinized gingiva increases slightly following GTR using bioresorbable membranes. This increase, however, appears to be smaller than for nonresorbable membranes, several studies actually reporting no effect or decreased keratinized gingival post-treatment (Danesh-Meyer and Wikesjo, 2001).

Studies comparing GTR and subepithelial connective tissue graft suggest that both protocols offer means of obtaining root coverage of gingival recession defects. It appears, however, that the subepithelial connective tissue graft protocol provides improved root coverage over that observed following GTR. The subepithelial connective tissue graft protocol also results in a substantially increased KG compared to only incremental improvements following GTR. A possible explanation for these observations may be the occurrence of membrane exposures and ensuing compromised wound healing following GTR (Danesh-Meyer and Wikesjo, 2001).

In a meta-analysis on forty studies, Al-Hamdan et al. (2003) revealead that guided tissue regeneration-based root coverage resulted in an average of 74% recession depth reduction, 41% complete root coverage, 3 mm CAL gain, and 1 mm keratinized gingival gain. Both guided tissue regeneration-based root coverage and conventional mucogingival surgery produced significant (P<0.05) improvement compared to baseline measurements. Compared to guided tissue regeneration-based root coverage, conventional mucogingival surgery resulted in significantly (P<0.05) increased keratinized gingiva (2.1 mm vs. 1.1 mm), root coverage (81% vs. 74%), and percentage of defects with complete root coverage (55% vs. 41 %). Use of absorbable membranes, root conditioning, shallow pretreatment recession (< 4 mm), and corporate sponsorship all resulted in significantly (P<0.05) improved percentages of sites with complete root coverage but had no effect on other parameters.

4.1.4. Pedicle Soft Tissue Graft Procedures Combined with Enamel Matrix Proteins

Enamel matrix derivative (EMD; Emdogain; Biora AB, Malmö, Sweden), harvested from embryonic porcine teeth, has been extensively studied in animals and humans and has been also proposed to be used in the treatment of root coverage ((Berlucchi et al., 2002, 2005;

Abbaset al., 2003; Hägewald S, et al., 2002; McGuire and Nunn, 2003; Nemcovsky et al., 2004;Cueva et al., 2004; Modica et al., 2000).

The technique as described by Berlucchi et al. (2005) is as follows:

An intrasulcular incision is made, under local anesthesia, on the buccal aspect of the gingiva. The incision is extended horizontally up to one or two teeth mesially and distally to the tooth involved in order to mobilize the flap, avoiding vertical releasing incisions to preserve as much blood supply as possible. A full thickness flap was then elevated beyond the mucogingival junction; next, a partial thickness flap is elevated in order to mobilize the flap, ensuring a passive coronal adaptation 1 to 2 mm above the cemento-enamel junction.

Afterwards, the papillae adjacent to the involved tooth were de-epithelialized and a sling suture is placed, but left untied, mesially and distally to the recession. Then, the root surface is conditioned with an ethylenediamine-tetraacetic acid (EDTA) gel 24% for 2 minutes, in accordance with the manufacturer's indication, and rinsed with saline solution. EMD is applied on the conditioned root surface and the suture is tied, positioning the flap 1 to 2 mm above the cemento-enamel junction. Single or sling sutures are used to secure the other papillae (Berlucchi et al., 2005).

Cheng et al. (2007) reviewed coronally positioned flap, coronally positioned flap + chemical root surface conditioning, or coronally positioned flap + enamel matrix derivative (EMD) for the treatment of Miller class I and II gingival recession. Clinically, the present analysis demonstrated that all three groups are useful in treating Miller's class I and II recession defects. All three groups achieved considerable root coverage and gains in clinical attachment, and maintained the amount of keratinized tissue and shallow probing pocket depths. The application of EMD to denuded root surfaces treated with the coronally positioned flap procedure significantly increased the percentage of root coverage and the attachment level compared with coronally positioned flap alone and the coronallypositioned flap + chemical root surface conditioning procedure. In thepresent review, the coronally positioned flap and coronally positioned flap + chemical root surface conditioning groups resulted in root coverage percentage values ranging from 55 to 75%. The mean root coverage percentage of coronally positioned flap + EMD-treated sites ranged from 71.7 to 95.1%.

The average root coverage of coronally positioned flap plus EMD amounted to 84.33 ± 7.72% after 6 mo and 84.42 ± 8.75% at 12 mo. The outcome of coronally positioned flap + EMD was better than coronally positioned flap alone after 6 months (74.12 ± 15.80%) and 12 months (79.00 ± 0.00%). The amount of root coverage obtained was quite stable between 6 and 12 mo in the coronally positioned flap + EMD group for root coverage. This suggests that root coverage procedures in the coronally positioned flap alone and coronally positioned flap + chemical root surface conditioning procedures were unpredictable. They became more predictable when the coronally positioned flap procedure was improved by the modification of adding EMD (Cheng et al., 2007).

4.2. Free Soft Tissue Grafts

The autogenous free soft tissue graft procedures may be performed as (1) an epithelized graft or (2) a subepithelial connective tissue graft (non-epithelized graft), both usually taken from the area of the masticatory mucosa in the palate.

4.2.1. Epithelialized Soft Tissue Graft

A free soft tissue graft of masticatory mucosa is usually selected when there is no acceptable donor tissue present in the area adjacent to the recession defect or when a thicker marginal tissue is desirable. The procedure can be used for the treatment of a single tooth as well as for groups of teeth (Wennström and Pini Prato, 1997). The graft can be nonsubmerged: that is, placed on the surface of the recipient bed; or submerged, when the graft is completely or partially covered by flap (Bouchard et al., 2001).

The epithelialized soft tissue graft is commonly named free gingival graft. The procedure can be performed either as a one-step technique , in which the graft is placed directly over the root surface either as a two-step surgical technique, where an epithelialized free soft tissue graft is placed apical to the recession and following healing is coronally positioned over the denuded root (Wennström et al., 2008).

The characteristics of the incision at the recipient site are important as means to optimize blood supply to the graft. Horizontal and vertical incisions should be made at a 90^0 angle, in a butt joint fashion. Beveled incisions may result in a tendency for the graft to slide over the incision lines with resultant dead space between the graft and the graft bed and, therefore, blood supply may be compromised. The vertical incisions in the recipient site should be placed close to the line angles of the adjacent teeth in order for wide surgical papillae to be present and consequently facilitate suturing and maximize blood supply from the papillary areas (Figures 5 and 6.) (Camargo et al., 2001).

As a matter of fact, graft thickness should be considered as an important criteria and should be controlled carefully. The grafts used should be approximately 0.8 to 1.5 mm in thickness to assure that there is an adequate connective tissue component (Kassab et al., 2010). However, deep wounds at the donor site may be created while receiving a transplant tissue from the palatal donor site. This donor region may be a source of arterial injury. In addition, an unaesthetic bulky tissue profile may also occur at the recipient site. On the other hand, very thin grafts (0.5 to 0.6 mm thickness) demonstrate a better color blending with that of the neighboring tissues (Hatipoğlu et al., 2007 with references therein).

After utilization of a free soft tissue graft, the vestibular depth of the recipient area may be diminished by the contraction of the wound and by the reinsertion of the muscle fibers in postoperative stage. Different clinical studies presented a broad range of shrinkage percentages between 12% and 48% (Hatipoğlu et al., 2007 with references therein).

Silva et al. (2010) sought to determine the effect of smoking on free soft tissue graft donor-site healing. A significantly lower proportion of smokers exhibited immediate bleeding after graft harvesting compared to non-smokers. Non-smokers had almost twice as long median time to achieve hemostasis compared to smokers in However, at 15 days 92% of nonsmokers sites and only 20% of smokers demonstrated complete epithelialization. At 30 days after the surgery, all sites in both groups demonstrated complete epithelialisation (Silva et al., 2010).

The free gingival graft used for root coverage presents distinct advantages over other surgical techniques, but also has its limitations. With appropriate case selection, this technique is predictable in achieving complete root coverage. The free gingival graft appears to be the best treatment alternative in areas where gingival recession is combined with lack of adequate vestibular depth and for teeth requiring root coverage prior to receiving a restoration with subgingival margins (Camargo et al., 2001).

An overview of studies on the effect of the free soft tissue graft as a means for root coverage was performed by Wennström (1996). The mean initial depth of the recessions included was 2.1 mm to 5.1 mm. The mean percent root coverage obtained with the free soft tissue graft procedure varied between 11% and 87%, with the greatest success in narrow and shallow defects. Considering the number of teeth treated in each study, the calculated average percentage of root coverage studies is 72%. The predictability of complete root coverage ranged from 0% to 90%, with an average of 57%.

The limitations and disadvantages of the free gingival graft for root coverage include increased discomfort and potential for postoperative bleeding from the donor area by virtue of a large wound that heals by secondary intention (Wessel and Tatakis, 2008; Del Pizzo et al., 2002).The palatal surgical wound heals with secondary intention within 2–4weeks (Farnoush 1978) due to the removal of the epithelial layer of the palatal mucosa. Compared with other soft tissue techniques for root coverage, the free gingival graft results in an unpredictable color match between the grafted tissue and adjacent gingival tissues. Grafted tissue with a lighter color than desired may persist for long periods of time after the initial healing. Finally, this procedure is technique sensitive and attention to the details involved in the execution of the surgery is crucial in order to achieve a successful outcome (Camargo et al., 2001; Kerner et al., 2009).

4.2.2. Connective Tissue Graft

The technique utilizing a subepithelial soft tissue graft, i.e. the connective tissue, involve the placement of the graft directly over the exposed root and the mobilization of a mucosal flap coronally or laterally for coverage of the graft (Wennström et al., 2008).

The most common indications for the CTG are esthetic demands from the patients, Miller Class I and II recession, dental hypersensitivity because of exposed cementoenamel junction and the necessity to augment a narrow band of keratinized gingival tissue. The relative contraindications that may limit the results of the connective tissue autograft are heavy smoking, impaired healing response from the patient, Miller Class II I or IV recession, or the existence of an extremely thin periodontium that would limit the amount of donor tissue (Zabalegui et al., 1999).

The tehnique of connective tissue graft covered by a coronally advanced flap(Wennström and Pini Prato 1997) is as follows:

- A horizontal incision is first made in the facial surface of the interdental tissue on each side of the teeth to be treated. The incision should be placed just coronal to the intended level of root coverage. Care should be taken not to decrease the height of the papilla. Subsequently, starting from the line of incision in the interdental area at the mesial and distal termination of the surgical area, two divergent, vertical incisions are placed and extended well beyond the mucogingival line.
- A split thickness flap is then prepared by sharp dissection and elevated to such an extent that it can be coronally repositioned at the level of the cementoenamel junction without tension.
- A subepithelial connective tissue graft of masticatory mucosa is harvested on the palatal aspect of the maxillary premolars (or from retromolar pad) by the use of a "trap door" approach. Before incisions are placed, the available thickness of the

mucosa is estimated by the use of the type of the syringe. A horizontal incision, perpendicular to the underlying bone surface, is made approximately 3mm apical to the soft tissue margin in the premolar region. The mesiodiostal extension of the incision is determined by the graft size required. To facilitate the removal of the graft, a vertical releasing incision can be made at the mesial termination of the primary incision. An incision is then placed from the line of the first incision and directed apically to perform a split incision of the palatal mucosa. A small periosteal elevator is used to release the connective tissue graft. Sutures may be placed in the graft before it is released completely free from the donor area to facilitate is placement at the recipient site.

- The graft is immediately placed in the recipient site and secured in position with interrupted sutures. The mucosal flap is then sutured to cover the connective tissue graft. Interrupted sutures are placed in the papilla region as well as along the wound of the vertical incisions. It is recommended to place a surgical dressing for protection of the area during the first week of healing.
- It has been showed that the clinical outcome of this surgical method is not affected by orientation of connective tissue graft (Laftzi et al., 2007; Al-Zahrani et al., 2004) nor by the presence of the epithelial collar (Byun et al. 2004).

Bouchard et al. (2001) performed an evaluation of 16 studies on the effect of free connective tissue grafts in the treatment of recession defects. The maximum length of the selected studies was 18 months. The mean initial depth of the treated recessions was 3.9 mm (3.3-4.9mm) for submerged grafts followed by rotational flaps the mean % of root coverage (range) was 83% (70-97). When considering the submerged grafts followed by coronally positioned flap at a mean initial depth of the treated recessions of 4.0 mm (3.0-5.6 mm), the mean % of root coverage (range) was 82% (52-99).

Chambrone et al. (2008) evaluated the effectiveness of subepithelial connective tissue grafts over other techniques when used in the treatment of recession defects, in terms of changes in clinical outcomes, occurrence of adverse effects, aesthetic condition and patient's satisfaction. The results indicated a statistically significant greater reduction in gingival recession for subepithelial connective tissue grafts, when compared to acellular dermal matrix graft (Weighted mean difference -0.63mm; 95% CI: -1.26, 0.00) and guided tissue regeneration with resorbable membranes(Weighted mean difference -0.41mm; 95% CI: -0.62, -0.20). For clinical attachment level changes, differences in CAL gain between all groups were not significant. For changes in the width of keratinized tissue, the results showed a statistically significant gain in the width of keratinized tissue for subepithelial connective tissue grafts when compared to guided tissue regeneration with resorbable membranes (Weighted mean difference -1.46mm; 95% CI: -2.12, -0.81), guided tissue regeneration with non-resorbable membranes (Weighted mean difference -1.82mm; 95% CI: -3.28, -0.35) and guided tissue regeneration with resorbable membranes associated to bone substitutes (Weighted mean difference -2.10mm; 95% CI: -2.51, -1.69). The percentages of compete root coverage and mean root coverage showed markedly variation. Procedures of subepithelial connective tissue grafts have given 8.6% - 96.1% complete root coverage and 64.5% - 97.3% mean root coverage. Overall comparisons allowed the authors to consider subepithelial connective tissue graft as the "gold standard" procedure in the treatment of recession-type defects.

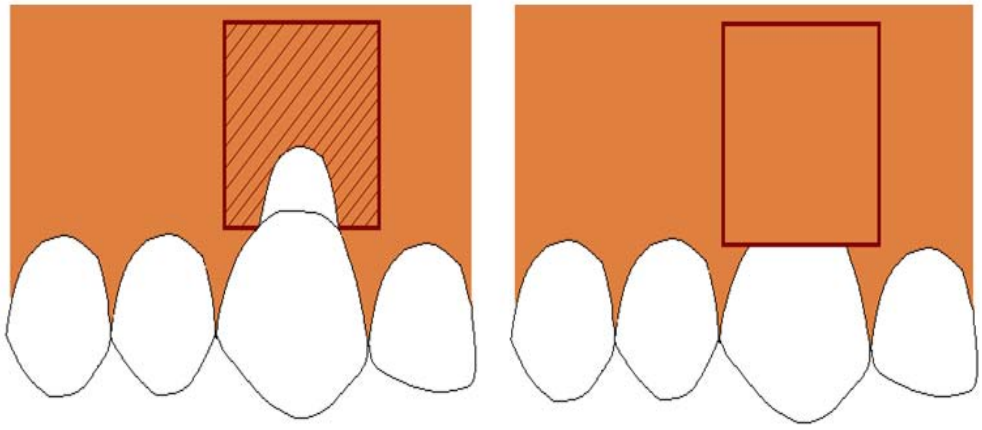

Figure 5. Schematic drawing of free gingival graft.

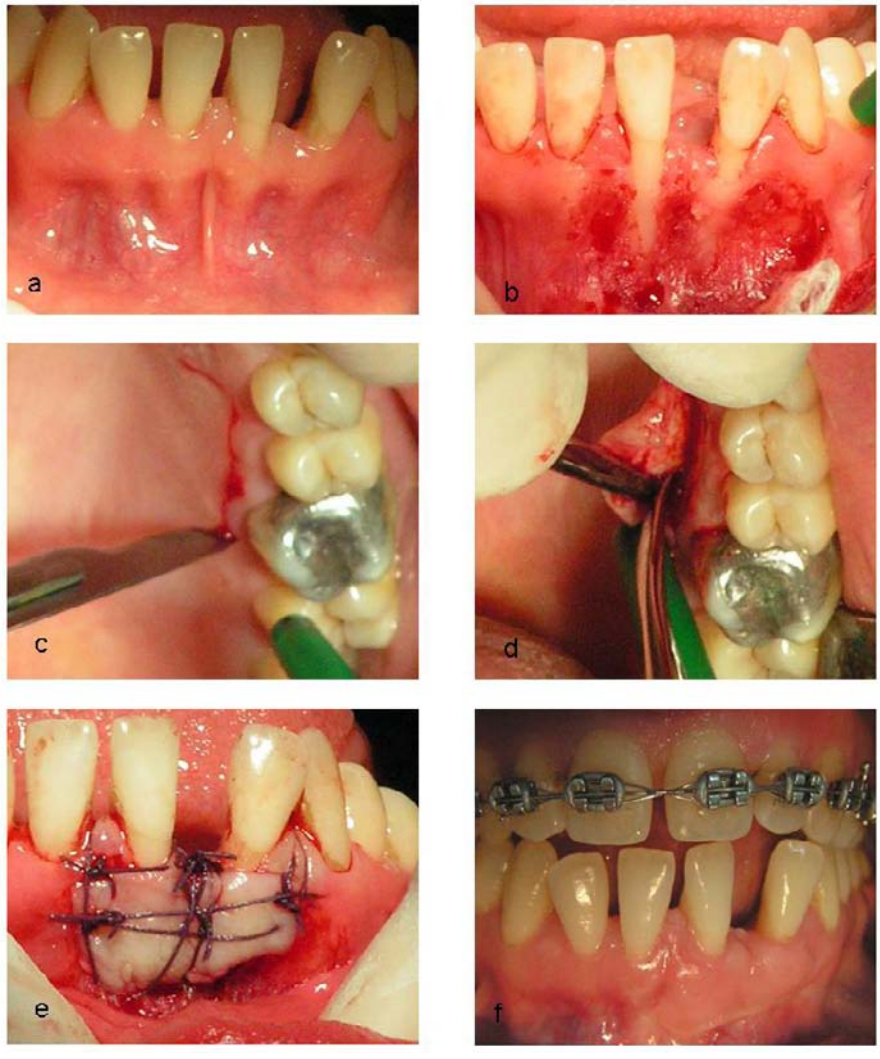

Figure 6. Epithelialized free soft tissue graft procedure.

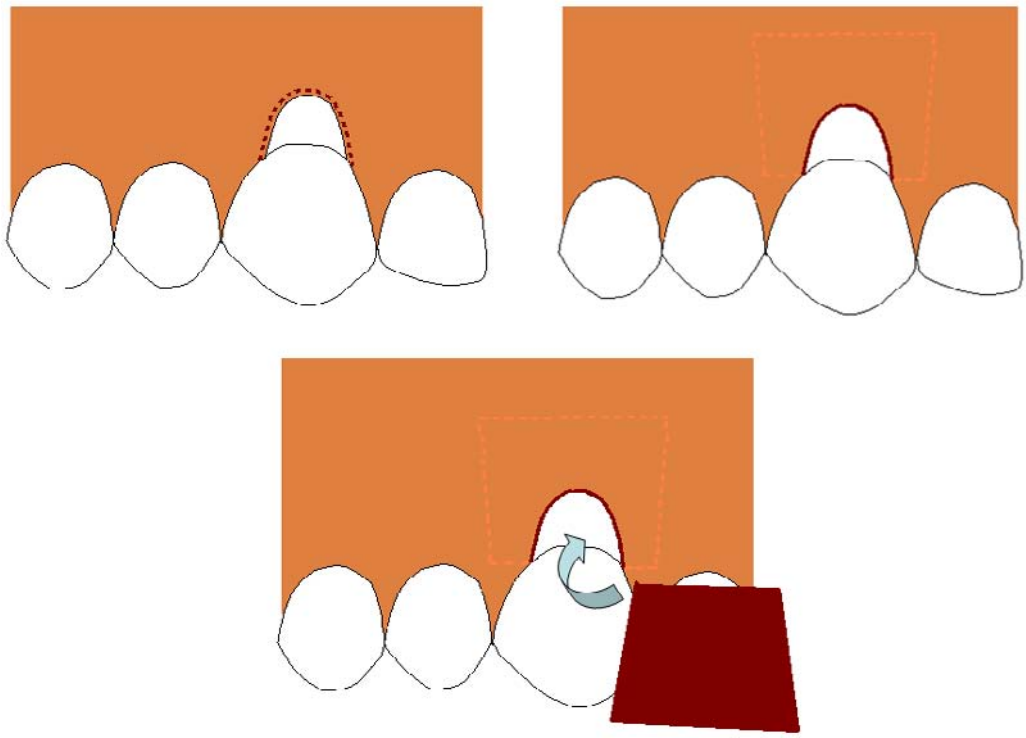

Figure 7. Schematic drawing of the “envelope” technique.

Subepithelial connective tissue grafts have been showed to be statistically superior to guided tissue regenerationwith resorbable membranes in achieving root coverage. Acellular dermal matrix grafts were proposed as an alternative in cases where subepithelial connective tissue grafts harvested from the palate are not sufficient to cover a recession area (Chambrone et al., 2009).

The “Envelope” Technique

Several modifications have been developed in managing the connective tissue (CT) graft.Raetzke (1985) demonstrated an ‘‘envelope’’ technique with no releasing incisions to secure the donor CT into an envelope created around the denuded root surface of a single gingival recession defect.

The sulcular epithelium of the affected tooth is removed and the exposed root is thoroughly scaled and planed followed by treatment with citric acid. A partial thickness envelope is created in the tissues surrounding the recession. A graft twice the width of the area of recession is placed into the envelope, completely covering the exposed root. Finger pressure is then applied to stabilize the graft until hemostasis is achieved. Tissue adhesive is used to keep the graft in place rather than sutures (Sedon et al., 2005) (Figure 7.)

Cordioli et al. (2001) evaluate root coverage and mucogingival changes 1 to 1.5 years following treatment of Miller's Class I and II recession defects using 2 variants of the subepithelial connective tissue graft procedure. Results showed a mean root coverage percentage of 89.6 ± 15% for the envelope technique group and 94.7 ± 11.4% for the coronally positioned flap combined with connective tissue graft group; the difference between

groups was statistically insignificant (P>0.05). Mean keratinized tissue increased significantly from 1.4 ± 1.1 mm presurgery to 4.5 ± 1.1 mm postsurgery for the envelope technique group while a minor increase in KT was observed in the coronally positioned flap combined with connective tissue graft group (2 ± 1.5 mm presurgery versus 2.7 ± 1.6 mm postsurgery) (Cordioli et al., 2001). For an mean initial recession depth of 2.9 mm (2.5-3.4), an mean % root coverage of 83% (80-87) has been reported (Bouchard et al., 2001). Among four studies reviewed(Allen, 1994; Jepsen et al., 1998; Müller et al., 1998; Raetzke, 1985) , the % teeth with complete root coverage was 53 (42–62) (Bouchard et al., 2001).

The key to the envelope flap is that it preserves the lateral and apical blood supply of the flap by eliminating vertical release incisions (Sedon et al., 2005). This technique minimizes surgical trauma to the recipient bed and provides good healing and excellent esthetic results. Among various connective tissue graft procedures, it is the last conducive to complete coverage of the defect by the overlying tissue, because of the manner in which the recipient bed is prepared (Yotnuengnit et al., 2004). In general, this surgical method provided excellent root coverage and an increased amount of keratinized gingiva.Vergara and Caffesse (2004) reported that thecomplete root coverage mean was 85%, 65%, and 16% for recession Class I, II, and IV, respectively.

The “Tunnel” Technique

The treatment of multiple adjacent gingival recessions with a tunnel subepithelial connective tissue graft has been proposed (Zabalegui et al., 1999). The surgical procedure involves a connective tissue graft placed in a multienvelope recipient bed (tunnel). This tunnel is made of a supraperiosteal bed under a pedicle flap without any external incisions. A connective tissue graft is then placed and secured through this tunnel, covering the adjacent exposed roots. The specific indications for surgical intervention with the tunnel CTG include multiple adjacent recessions, situations in which very early healing is needed for esthetic demands, or a need to reduce the number of surgical interventions (Zabalegui et al., 1999).

Tözüm et al. (2005) compared the efficiency of two different modified tunnel technique with the Langer and Langer, in Miller Class I and II gingival recessions. Langer and Langer (1985) described the root coverage technique in which the overlying partial thickness flap with two vertical incisions covers the transplanted connective tissue graft. Both techniques demonstrated highly predictable root coverage and attachment gain at 6 months post-surgery, but better results were obtained for the tunnel technique for root coverage and attachment gain. A mean root coverage of 3.36 ± 0.17 mm and 3.93 ± 0.27 mm attachment gain were noted in the tunnel group compared to the Langer and Langer group, where a mean root coverage of 2.56 ± 0.19 mm (P<0.005) and 2.44 ± 0.34 mm (P<0.005) attachment gain were achieved. The percentage of root coverage was 96.4% and 75.5% and attachment gain was 77.1% and 56.4% in the tunnel and Langer and Langer groups, respectively.

Subepithelial connective tissue grafts with modified tunnel approach have showed also long-term stability of the results (Tözüm, 2006; Ribeiro et al., 2008). It was reported a mean root coverage was 95% and 92.2% at eight months and 36 months postsurgery, respectively. These differences were statistically significant compared to the baseline. The mean gain in attachment was 3.79 mm, and the mean root coverage was 3.14 mm after 36 months (Tözüm, 2006).

The tunnel technique offers successful clinical results for both patients and clinicians, as preservation of the interdental papillae reduce the trauma at the recipient site and improve early esthetic results (Tözüm, 2003).

5. Factors Influencing the Success of Root Coverage Procedures

5.1. Patient Characteristics

Gingival recession is often a source of anxiety to patients. Noncompliant patients should be considered at risk. It was suggested that the clinician should carefully assess patient's expectations and motivation for seeking treatment (Grey, 2000).

An unsatisfied patient already subjected to multiple aesthetic procedures should be suspected of never being satisfied. The demand for repeated surgery can be, in fact, a sign of psychopathology (polysurgical addiction). Patients presenting factitious gingival ulceration should be carefully evaluated prior to root coverage procedures (Bouchard et al., 2001).

Patients with poor oral hygiene who are prone to periodontal destruction are also at great risk for surgical failure unless the local factors can be controlled (Grey, 2000).

In addition to clinical outcomes, another aspect to be considered is the possible change in the soft tissues caused by smoking. A recent meta-analysis performed by Chambrone et al. (2009) indicated a statistically significant greater reduction in gingival recession (P <0.001) and gain in clinical attachment level (P < 0.001) for nonsmokers when compared with smokers whose gingival recession was treated with subepithelial connective-tissue grafts. Additionally, nonsmokers exhibited significantly more sites with complete root coverage than did smokers (P = 0.001). Subepithelial connective-tissue grafts resulted in 27.0 to 80.0% complete root coverage for nonsmokers and 0 to 25.0% for smokers. Similarly, coronally advanced flaps resulted in 20.0 to 55.1% complete root cov erage for nonsmokers and 0 to 54.5% for smokers. For guided tissue regeneration, complete root coverage was 38.5% for nonsmokers and 11.1% for smokers. Between smokers and nonsmokers who received subepithelial connective-tissue grafts, nonsmokers achieved more complete root coverage. They showed a significant difference in the number of sites with complete root coverage when compared with smokers (risk ratio, 0.24; 95% CI: 0.10 to 0.58) in the two arms of the trials.

Similar results were revealed by Souza et al. (2008) who showed that smoking can reduce the root coverage obtained with an SCTG associated with a coronally positioned flap. The percentages of root coverage in smokers after 3 months (62.10% ± 19.08%) and 6 months (58.02% ± 19.75%) were substantially lower than that of non-smokers (82.17% ± 16.47% and 83.35% ± 18.53%, respectively).

5.2. Anatomic Features

In the literature, gingival recessions have been classified into four classes, according to the prognosis of root coverage. In Class I and II gingival recessions, there is no loss of

interproximal periodontal attachment and bone and complete root coverage can be achieved; in Class III, the loss of interdental periodontal support is mild to moderate, and partial root coverage can be accomplished; in Class IV, the loss of interproximal periodontal attachment is so severe that no root coverage is feasible. More recently, other factors than the level of interproximal attachment and bone have been shown to limit the amount of root coverage: the reduction of papilla height, tooth rotation and tooth extrusion with or without occlusal abrasion. In all these clinical situation, only partial root coverage can be achieved (Zucchelli et al., 2010).

The smile line also needs to be considered. Normally, the cosmetic zone is limited to the maxilla. Patients presenting a ‘‘gummy smile’’ should be carefully evaluated before root coverage procedures. The surgical challenge is great, because the smile will expose the entire operated zone. These patients may require orthodontics and orthognatic surgery to improve the lip line (Bouchard et al., 2001).

5.3. Technique Characteristics

Periodontal plastic surgery is an art as much as an science and a skilled practitioner can obtain more satisfactory results than those with less skills and experience (Grey, 2000). In periodontal plastic surgery, the choice of procedure is based on the four cardinal principles of any surgery: success, reproducibility, lack of morbidity and economy. Basically, the easier the technique the more reproducible it is, since the need for technical skill of the surgeon is reduced. The surgeon’s choice will be based on the confidence he has of his own ability to match the outcomes of the clinical trials (Bouchard et al., 2001).

Criteria for selection of techniques are (Takei et al. 2006):

1. Surgical site free of plaque, calculus, and inflammation
2. Adequate blood supply to the donor site
3. Anatomy of the recipient and donor site (vestibulat depth, width of keratinized gingival, palatal tissue thickness)
4. Stability of the grafted tissue to the recipient site
5. Minimal trauma to the recipient site

Several technique-related factors may influence the treatment outcomes:

- The flap thickness. Thick gingival tissue eases manipulation, maintains vascularity, and promotes wound healing during and after surgery. Significant moderate correlation occurred between weighted flap thickness andweightedmean root coverage and weighted complete root coverage ($r = 0.646$ and 0.454, respectively). A critical threshold thickness >1.1 mm existed for complete root coverage ($P < 0.02$) (Hwang and Wang 2006)
- Elimination of flap tension is considered an important factor for the outcome of the coronally advanced flap procedure (Wennström et al., 2008; Greenstein et al., 2009).

- The position of the gingival margin relative to the cemento-enamel junction after suturing affects the probability of complete root coveragefollowing healing (Wennström et al., 2008).

Brouchard et al. (2001) revealed several outdated procedures: Nonsubmerged grafts are no longer justified in the coverage of recession defects for aesthetic purposes. The procedure is uncomfortable for the patient because of the denuded palatal donor site, and the match with the surrounding tissues is unpredictable. The double papilla flap also seems to be a dated technique. Use of elaborate sutures is time-consuming. The procedure requires surgeon's dexterity. Sutures placed over the avascular root surface may lead to postoperative cleft complications that may impair esthetic results. Similarly, there seems to be little clinical advantage in using double pedicle flap to cover connective tissue grafts (Brouchard et al., 2001).

References

AbbasF, WennströmJ, Van der WeijdenV, SchneidersT, Van der VeldenU. Surgical treatment of gingival recessions using emdogain gel: clinical procedure and case reports. *Int. J. Periodont. Restor. Dent.*2003;23:607–613.

Albandar JM, Kingman A. Gingival recession*J. Periodontol*. 1999;70:30-43.

Al-Hamdan K, Eber R, Sarment D, Kowalski C, Wang HL. Guided tissue regeneration-based root coverage: meta-analysis. *J. Periodontol.*2003;74:1520-33.

Allen AL. Use of the supraperiosteal envelope in soft tissue grafting for root coverage. II. Clinical results. *Int. J. Periodontics Restorative Dent.* 1994;14:302–315.

Allen EP, Miller PD Jr. Coronal positioning of existing gingiva: short term results in the treatment of shallowginal tissue recession. *J. Periodontol.* 1989;60:316-9.

Al-Zahrani MS, Bissada NF, Ficara AJ, Cole B. Effect of connective tissue*Int. J. Periodontics Restorative Dent.* 2004;24:65-9.

Anderegg CR, Metzler DG, Nicoll BK. Gingiva thickness in guided tissue*J. Periodontol.* 1995;66:397-402.

Bahat O, Handelsman M, Gordon J. The transpositioned flap in mucogingival surgery. *Int. J. Periodontics Restorative Dent.* 1990;10:472-82.

Baldi C, Pini-Prato G, Pagliaro U, Nieri M, Saletta D, Muzzi L, Cortellini P. Coronally advanced flap procedure for root coverage. Is flap thickness a relevant predictor to achieve root coverage? A 19-case series. *J. Periodontol.* 1999;70:1077-84.

Barriviera M, Duarte WR, Januario AL, Faber J, Bezerra AC. A new method to assess and measure palatal masticatory mucosa by cone-beam computerized tomography. *J. Clin. Periodontol.*2009;36:564-568.

Berlucchi I, Francetti L, Del Fabbro M, Basso M, Weinstein RL. The influence of anatomical features on the outcome of gingival recessions treated with coronally advanced flap and enamel matrix derivative: a 1-year prospective study. *J. Periodontol.* 2005;76:899–907.

Berlucchi I, Francetti L, Del Fabbro M, Testori T, Weinstein RL. Enamel matrix proteins (Emdogain) in combination with coronally advanced flap or subepithelial connective

tissue graft in the treatment of shallow gingival recessions. *Int. J. Periodont. Restor. Dent*. 2002;22:583–593.

Bernimoulin JP, Luscher B, Muhlemann HR. Coronally repositioned periodontal flap. Clinical evaluation after one year. *J. Clin. Periodontol*. 1975;2:1-13.

Bittencourt S, Del Peloso Ribeiro E, Sallum EA, Sallum AW, Nociti FH Jr, Casati MZ. Comparative 6-month clinical study of a semilunar coronally positioned flap and subepithelial connective tissue graft for the treatment of gingival recession. *J. Periodontol*. 2006;77:174-81.

Bittencourt S, Ribeiro Edel P, Sallum EA, Sallum AW, Nociti FH, Casati MZ. Semilunar coronally positioned flap or subepithelial connective tissue graft for the treatment of gingival recession: a 30-month follow-up study. *J. Periodontol*. 2009;80:1076-82.

Bouchard P, Malet J, Borghetti A.ision-making in aesthetics: root coverage revisited. *Periodontol. 2000* 2001;27:97-120

Byun HY, Oh TJ, Abuhussein HM, Yamashita J, Soehren SE, Wang HL. Significance of the epithelial collar on the subepithelial connective tissue*J. Periodontol*. 2009;80:924-32.

Cairo F, Pagliaro U, Nieri M. Treatment of gingival recession with coronally advanced flap procedures: a systematic review. *J. Clin. Periodontol*. 2008;35:136-162.

Camargo PM, Melnick PR, Kenney EB. The use of free gingival grafts for aesthetic purposes. *Periodontol 2000*. 2001;27:72-96.

Chambrone L, Chambrone D, Pustiglioni FE, Chambrone LA, Lima LA. Can subepithelial connective tissue grafts be considered the gold standard procedure in the treatment of Miller Class I and II recession-type defects? *J. Dent*. 2008;36:659-71.

Chambrone L, Chambrone D, Pustiglioni FE, Chambrone LA, Lima LA. The influence of tobacco*J. Am. Dent. Assoc*. 2009;140:294-306.

Chambrone L, Sukekava F, Araújo MG, Pustiglioni FE, Chambrone LA, Lima LA. Root coverage procedures for the treatment of localised recession-type defects. *Cochrane Database Syst. Rev*. 2009 15;:CD007161.

Cheng Y-F, Chen J-W, Lin S-J, Lu H-K. Is coronally positioned flap procedure adjunct with enamel matrix derivative or root conditioning a relevant predictor for achieving root coverage? A systemic review. *J. Periodont. Res*. 2007;42:474-485.

Claffey N, Shanley D. Relationship of gingival thickness and bleeding to loss of probing attachment in shallow sites following nonsurgical periodontal therapy. *J. Clin. Periodontol*. 1986;13:654-657.

Cohen DW, Ross SE. The double papillae repositioned flap in periodontal therapy. *J. Periodontol.*1968;39:65-70.

Cordioli G, Mortarino C, Chierico A, Grusovin MG, Majzoub Z. Comparison of 2 techniques J. Periodontol. 2001;72:1470-6.

Cueva MA, Boltchi FE, Hallmon WW, Nunn ME, Rivera-Hidalgo F, Rees T. A comparative study of coronally advanced flaps with and without the addition of enamel matrix derivative in the treatment ofginal tissue recession. *J. Periodontol.*2004;75:949-956.

Danesh-Meyer MJ, Wikesjö UM. Gingival recession defects and guided tissue regeneration: a review. *J. Periodontal Res*. 2001;36:341-54.

De Rouck T, Eghbali R, Collys K, De Bruyn H, Cosyn J. The gingival biotype revisited: Transparency of the periodontal probe through the gingivalgin as a method to discriminate thin from thick gingiva. *J.Clin. Periodontol*. 2009;36:428-433.

De Rouck T, Eghbali R, Collys K, De Bruyn H, Cosyn J. The gingival biotype revisited: transparency of the periodontal probe through the gingival margin as a method to discriminate thin from thick gingiva. *J. Clin. Periodontol*. 2009;36:428-33.

de Sanctis M, Zucchelli G. Coronally advanced flap: a modified surgical approach for isolated recession-type defects: three-year results. *J. Clin. Periodontol*. 2007;34:262-8.

Del Pizzo M, Modica F, Bethaz N, Priotto P, Romagnoli R. The connective tissue graft: a comparative clinical evaluation of wound healing at the palatal donor site. A preliminary study. *J. Clin. Periodontol.* 2002;29:848-854.

Espinel MC, Caffesse RG. Comparison of the results obtained with the lateral positioned pedicle sliding flap revised technique and the lateral sliding flap with a free gingival graft technique in the treatment of localized gingival recession. *Int. J. Periodontics Restorative Dent.*1981;1:30-37.

Evans CD, Chen ST. Esthetic outcomes of immediate implant placement*Clin. Oral. Implants Res*. 2008;19:73-80.

Farnoush A. Techniques for protection and coverage of the donor sites in free soft tissue graft. *J. Periodontol.* 1978;49:403-405.

Fu JH, Yeh CY, Chan HL, Tatarakis N, Leong DJ, Wang HL. Tissue biotype and its relation to the underlying bone morphology. *J. Periodontol*. 2010;81:569-74.

Gray JL. When not to perform root*J. Periodontol*. 2000;71:1048-50.

Greenberg J, Laster L, Listgarten MA. Transgingival probing as a potential estimator of alveolar bone level. *J. Periodontol*. 1976;47:514-517.

Greenstein G, Greenstein B, Cavallaro J, Elian N, Tarnow D. Flap advancement*J. Periodontol*. 2009;80:4-15.

Grupe HE, Warren RF. Repair of gingival defects by a sliding flap operation. *J. Periodontol*. 1956;27:290–5.

Guinard EA, Caffesse RG. Treatment of localized gingival recessions. Part III. Comparison of results obtained with lateral sliding and coronally repositioned flaps. *J. Periodontol*. 1978;49:457-461.

Hägewald S, Spahr A, Rompola E, Haller B, Heijl L, Bernimoulin JP. Comparative study of Emdogain and coronally advanced flap technique in the treatment of human gingival recessions. A prospective controlled clinical study. *J. Clin. Periodontol*. 2002;29:35–41.

Haghighat K. Modified semilunar coronally advanced flap. *J. Periodontol.* 2006;77:1274-9.

Harris RJ, Harris AW. - The coronally positioned pedicle graft with inlaidgins: a predictable method of obtaining root coverage of shallow defects. *Int. J. Periodontics Restorative Dent.* 1994;14:228-41.

Hatipoğlu H, Keçeli HG, Güncü GN, Sengün D, Tözüm TF. Vertical and horizontal dimensional evaluation of free gingival*Clin. Oral Investig*. 2007;11:107-13.

Huang LH, Neiva RE, Wang HL. Factors affecting the outcomes of coronally advanced flap root coverage procedure. *J. Periodontol*. 2005;76:1729-1734.

Hurzeler MB, Weng D. Functional and esthetic outcome enhancement of periodontal surgery by application of plastic surgery principles. *Int. J. Periodontics Restorative Dent*. 1999;19:36-43

Hwang D, Wang HL. Flap thickness as a predictor of root coverage: A systematic review. *J. Periodontol.* 2006;77:1625-1634.

Januário AL, Barriviera M, Duarte WR. Soft tissue*J. Esthet Restor. Dent*. 2008;20:366-73; discussion 374.

Jepsen K, Heinz B, Halben J, Jepsen S. Treatment of gingival recession with titanium reinforced barrier membranes versus connective tissue grafts. *J. Periodontol*. 1998;69:383-391.

Kao RT, Fagan MC, Conte GJ. Thick vs. thin gingival*J. Calif. Dent. Assoc*. 2008;36:193-8.

Kassab MM, Badawi H, Dentino AR. Treatment of gingival recession. *Dent. Clin. North Am*. 2010;54:129-40.

Kassab MM, Cohen RE. The etiology*J. Am. Dent. Assoc*. 2003;134:220-5.

Kerner S, Sarfati A, Katsahian S, Jaumet V, Micheau C, Mora F, Monnet-Corti V, Bouchard P. Qualitative cosmetic*J. Periodontol*. 2009;80:41-7.

Lafzi A, Mostofi Zadeh Farahani R, Abolfazli N, Amid R, Safaiyan A. Effect of connective tissue*Clin. Oral. Investig*. 2007;11:401-8.

Langer B, Langer L. Subepithelial connective tissue graft technique for root coverage. *J. Periodontol*. 1985;56:715-720.

McGuire MK, Nunn M. Evaluation of human recession defects treated with coronally advanced flaps and either enamel matrix derivative or connective tissue. Part 1: Comparison of clinical parameters. *J. Periodontol*. 2003;74:1110-1125.

McGuire MK. Periodontal plastic surgery. *Dent. Clin. North Am*. 1998;42:411-65.

Milano F. A combined flap for root coverage. *Int. J. Periodontics Restorative Dent*. 1998;18:544-51.

Miller PD Jr. A classification ofginal tissue recession. *Int. J. Periodontics Restorative Dent*. 1985;5:9-13.

Modica F, Pizzo MD, Roccuzzo M, Romagnoil R. Coronally advanced flap for the treatment of buccal gingival recessions with and without enamel matrix derivative. A split-mouth study. *J. Periodontol*. 2000;71:1693-1698.

Müller HP, Eger T, Schorb A. Gingival dimensions after root coverage with free connective tissue grafts. *J. Clin. Periodontol.* 1998;25:424-430.

Müller HP, Heinecke A, Schaller N, Eger T. Masticatory mucosa in subjects with different periodontal phenotypes. *J. Clin. Periodontol*. 2000;27:621-626.

Nemcovsky CE, Artzi Z, Tal H, Kozlovsky A, Moses O. A multicenter comparative study of two root coverage procedures: coronally advanced flap with addition of enamel matrix proteins and subpedicle connective tissue graft. *J. Periodontol.* 2004;75:600-607.

Ochsenbein C, Ross S. A concept of osseous surgery and its clinical applications. In: Ward HL, Chas C, eds. A periodontal point of view. Springfield, IL: Charles C. Thomas, 1973:2 76-322.

Oles RD, Ibbott CG, Laverty WD. Effects of citric acid treatment on pedicle flap coverage of localized recession.*Periodontol*. 1985;56:259-261.

Oles RD, Ibbott CG, Laverty WD. Effects of root curettage and sodium hypochlorite treatment on pedicle flap coverage of localized recession. *J. Can. Dent. Assoc*. 1988;54:515-517.

Patur B. The rotation flap for covering denuded root surfaces - a closed wound technique. *J. Periodontol*. 1977;48:41-4.

Pennel B, Higgason JD, Towner JD, King KO, Froitz BD, Salder JF. Oblique Rotated Flap. *J Periodontol*. 1965:36:305-309.

Raetzke PB. Covering localized areas of root exposure employing the ‘‘envelope’’ technique. *J. Periodontol*. 1985;56:397-402.

Ribeiro FS, Zandim DL, Pontes AE, Mantovani RV, Sampaio JE,cantonio E. Tunnel technique with a surgical maneuver to increase the graft extension: case report with a 3-year follow-up.*J. Periodontol*.2008;79:753-8.

Romanos GE, Bernimoulin JP,ggraf E. The double lateral bridging flap for coverage of denuded root surface: longitudinal study and clinical evaluation after 5 to 8 years. *J. Periodontol*. 1993;64:683-8.

Romeo E, Lops D, Rossi A, Storelli S, Rozza R, Chiapasco M. Surgical and prosthetic management*J. Periodontol*. 2008;79:1048-55.

Sanavi F, Weisgold AS, Rose LF. Biologic width and its relation to periodontal biotypes. *J. Esthet. Dent*. 1998;10:157-63.

Santana RB, Furtado MB, Mattos CM, de Mello Fonseca E, Dibart S. Clinical evaluation of single-stage advanced versus rotated flaps in the treatment of gingival recessions. *J. Periodontol*. 2010;81:485-92.

Sedon CL, Breault LG, Covington LL, Bishop BG. The subepithelial connective tissue graft: part I. Patient selection and surgical techniques. *J. Contemp. Dent. Pract.* 2005;6:146-62.

Seibert JL, Lindhe J. Esthetics and periodontal therapy. In: Lindhe J, ed. *Textbook of Clinical Periodontology*. 2nd ed. Copenhangen, Denmark: Munksgaard;1989:477-514.

Silva CO, Ribeiro Edel P, Sallum AW, Tatakis DN. Free gingival*J. Periodontol*. 2010;81:692-701.

Smith RG. Gingival recession. Reappraisal of an enigmatic condition and a new index for monitoring. *J. Clin. Periodontol*. 1997;24:201–5.

Smuckler H. Laterally positioned mucoperiosteal pedicle grafts in the treatment of denuded roots. A clinical and statistical study. *J. Periodontol*. 1976;47:590-595.

Souza SL, Macedo GO, Tunes RS, Silveira e Souza AM,aes AB Jr, Grisi MF, Taba M Jr, Palioto DB, Correa VM. Subepithelial connective tissue graft for root coverage in smokers and non-smokers: a clinical and histologic controlled study in humans. *J. Periodontol*. 2008;79:1014-21.

Staffileno H. Management of gingival recession and root exposure problems associated with periodontal disease. *Dent. Clin. North Am*. 1964: 111-120.

Sumner CF 3rd. Surgical repair of recession on the maxillary cuspid. Incisally repositioning the gingival tissues. *J. Periodontol*. 1969;40:119-121.

Takei HH, Azzi RR, Han TJ. Periodontal plastic and esthetic surgery. In *Carranza's Clinical Periodontology*by Newman MG, Takei H, Carranza FA, Klokkevold PR. Saunders; 10 edition, 2006: 1005-1029.

Tarnow DP. Semilunar coronally repositioned flap. *J. Clin. Periodontol*. 1986;13:182-5

Tözüm TF, Keçeli HG, Güncü GN, Hatipoğlu H, Sengün D. Treatment of gingival*J. Periodontol*. 2005;76:1842-8.

Tözüm TF. A promising periodontal procedure for the treatment of adjacent gingival recession defects. *J. Can. Dent. Assoc*. 2003;69:155-9.

Tözüm TF. Root coverage with subepithelial connective tissue*N. Y. State Dent. J*. 2006-Jul;72:38-41.

Trombelli L. Periodontal regeneration*Periodontol. 2000*. 1999;19:138-50.

Vergara JA, Caffesse RG. Localized gingival*J. Periodontol.* 2004;75:1397-403.

Waite IM. An assessment of the postsurgical results following the combined laterally positioned flap and gingival graft procedure. *Quintessence Int*. 1984;15:441-450.

Wennström J, Pini Prato GP. Mucogingival Therapy. In: LindheJ, ed. *Clinical periodontology and implant dentistry.* 3rd edn. Munksgaard: Copenhagen, 1997: 550-596

Wennström JL, Zucchelli G, Pini Prato GP. Mucogingival therapy – Periodontal plastic surgery. In Lindhe J,Lang NP, Karring T. *Clinical Periodontology and Implant Dentistry*. Wiley-Blackwell; 5 edition, 2008: 955-1029.

Wennström JL, Zucchelli G. Increased gingival dimensions. A significant factor for successful outcome of root coverage procedures? A 2-year prospective clinical study. *J. Clin. Periodontol*. 1996;23:770-777.

Wennström JL. Mucogingival surgery. In Lang NP, Karring T, Lindhe J, *Proceedings of the 2nd European Workshop on Periodontology*: Implant Dentistry, Quintessence Pub Co, 1997: 193- 209.

Wennström JL. Mucogingival therapy. *Ann. Periodontol*. 1996;1:671-701.

Wennström JL. The significance of the width and thickness of the gingiva in orthodontic treatment*Dtsch Zahnarztl Z*. 1990;45:136-41.

Wessel JR, Tatakis DN. Patient outcomes following subepithelial connective tissue graft and free gingival graft procedures. *J. Periodontol.* 2008;79:425-30.

Yotnuengnit P, Promsudthi A, Teparat T, Laohapand P, Yuwaprecha W. Relative connective tissue graft size affects root coverage treatment outcome in the envelope procedure. *J. Periodontol.* 2004;75:886-92.

Zabalegui I, Sicilia A, Cambra J, Gil J, Sanz M. Treatment of multiple adjacent gingival recessions with the tunnel subepithelial connective tissue graft: a clinical report. *Int. J. Periodontics Restorative Dent*. 1999;19:199-206.

Zade RM, Hirani SH. A clinical study of localized gingival recession treated by lateral sliding flap. *J Indian Dent Assoc.*1985;57:19-26.

Zigdon H, Machtei EE. The dimensions of keratinized mucosa around implants affect clinical and immunological parameters. *Clin. Oral. Implants Res.* 2008;19:387-392.

Zucchelli G, Cesari C, Amore C, Montebugnoli L, De Sanctis M. Laterally moved, coronally advanced flap: a modified surgical approach for isolated recession-type defects. *J. Periodontol*. 2004;75:1734-41.

Zucchelli G, De Sanctis M. Treatment of multiple recession type defects in patients with aesthetic demands. *J. Periodontol.2000*;71:1506-1514.

Zucchelli G, Mele M, Stefanini M, Mazzotti C, Mounssif I,zadori M, Montebugnoli L.Predetermination of root coverage. *J. Periodontol*. 2010 [Epub ahead of print]

Zucchelli G, Testori T, DeSanctis M. Clinical and anatomical factors limiting treatment outcomes of gingival recession: A new method to predetermine the line of root Coverage. *J. Periodontol.*2006;77:714-721.

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Chapter II

Periodontal Diseases in Children and Adolescents: Clinical Features and Molecular Biological Analyses

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Abstract

The clinical features of periodontal diseases in children and adolescents differ from those in adults. Periodontitis is extremely rare in children, except those complicated with certain kinds of systemic diseases, whereas gingivitis is commonly encountered. Childhood gingivitis can be reversed by professional mechanical tooth cleaning in combination with tooth brushing instruction. On the other hand,gingivitis becomes increasingly prevalent with age through the adolescent period, and early diagnosis and appropriate interventions are necessary to prevent the onset of marginal periodontitis during adolescence. Since most children with periodontitis possess a background of abnormal immune responses, they have a lower likelihood of good prognosis,even though diligent interventions are performed. Other types of periodontal diseases include gingival recession, which is mainly caused by traumatic occlusion, and gingival overgrowth, which has a hereditary background and is associated with specific medication such as antiepilepticphenytoin. In addition, cases with a rapid loss of gingival attachment and

alveolar bone due to mechanical injury at the periodontal sulcus, termed “acute periodontitis,” are also encountered. Furthermore, an unintentional attachment loss, when materials such as small plastic tubes being fitted to the teeth are inserted, is a unique type of periodontitis in young children. It should be noted that periodontitis associated with anatomical anomalies, which are derived from fragile periodontal attachment, is also encountered.

Considering the etiology of periodontitis, it is important to identify periodontitis-related bacterial species, since the disease is generally known to be caused by specific bacteria. However, most of those belong to the obligate anaerobic group, and it is difficult and time-consuming to isolate them. On the other hand, recent developments in molecular biological techniques have enabled rapid identification of species using bacterial DNA extracted from various kinds of clinical specimens. Such approaches do not require isolation of viable bacteria and even small amounts of DNA can be detected using PCR techniques. With such modern techniques, we have evaluated the distribution of periodontal bacterial species in children, changes of species in the same subjects over a long interval, combinations of species simultaneously detected, and mother-to-child transmission. In addition, the distributions of bacterial species in children with Down’s syndrome and other developmental disabilities have been analyzed. Our results have provided valuable information regarding bacterial profiles in clinical specimens, which should lead to further beneficial methods for clinical use in the near future.

1. Introduction

The clinical features of periodontal diseases in children and adolescents differ from those in adults. One of the major differences is that gingivitis is commonly encountered in children and that periodontitis is rare except for those who are complicated with certain types of systemic diseases. It is generally known that gingivitis is commonly seen in children and adolescents, and its prevalence, severity and extent tends to increase with age, beginning in the deciduous dentition stage and reaching a peak during puberty, followed by a limited decline in adolescence, whereas the loss of periodontal support due to periodontitis rarely occurs in the permanent dentition of teenage populations [1]. Other types of periodontal diseases include gingival recession, which is mainly caused by traumatic occlusion. In addition, gingival overgrowth with a hereditary background and with the association of specific medication is sometimes encountered. Furthermore, cases of rapid loss of gingival attachment and alveolar bone due to mechanical injury at the periodontal sulcus, which is called “acute periodontitis”, are encountered. Accidentally-induced periodontitis is unique for infants and young children, in which the materials fitting to the teeth, such as small plastic tubes, are accidentally inserted into the tooth crown and cause attachment loss. Cases of periodontitis associated with anatomical anomalies are also encountered and presence of the developmental groove can be one of the possible causes.

Periodontitis is generally an infectious disorder caused by complex actions of a small subset of periodontal bacteria. Since most of the species belong to the obligate anaerobes, special skills are required for isolation of the organisms from oral specimens. On the other hand, the recent development of molecular biological techniquesallows for the rapid identification of the species using bacterial DNA extracted from the various types of clinical specimens.

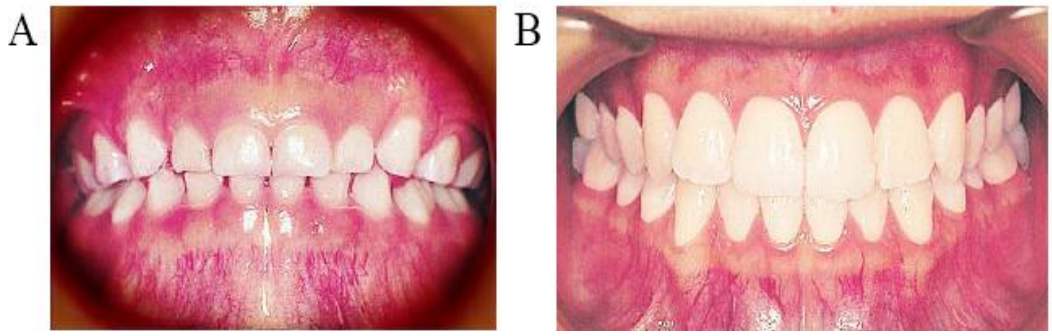

Figure 1. Typical features of healthy gingiva in the primary (A) and permanent (B) dentitions.

This approach does not require the isolation of the bacteria and even a small amount of bacterial DNA enables detection by the polymerase chain reaction (PCR) technique. Information regarding the bacterial species in oral specimens, such as saliva and dental plaque, has been accumulating since the latter half of 1990s. Such data has not only contributed to the analysis of the bacterial flora but also to the development of the beneficial methods for clinical use.

In this chapter, the clinical features of periodontal diseases in the pediatric dentistry field are described, followed by the presentation of the molecular biological data obtained from our analysis of various clinical specimens taken from Japanese children and adolescents.

2. Periodontal Diseases in Children and Adolescents

Healthy gingivae in children are soft and slightly red as compared to those of adults since the blood vessels in connective tissues are relatively transparent due to the loose collagen fibers under the thin keratinocyte layer (Figure 1). Stipplings in the gingiva are initially found at the age of 2-3 years and these become prominent at around 6-7 years old. The marginal gingiva appears to be round and thick, which is associated with the protruded morphology of the cervical areas in the primary teeth. The average periodontal pocket depths are approximately 1 mm for all teeth, whereas those of the maxillary primary molar regions tend to be slight deeper. When permanent teeth emerge inthe oral cavity, the gingival sulcusbecomes deeper and the marginal gingivae become extremely thin. The thickness of the marginal gingiva in children and adolescents increases to the same amount as adults by their late teens.

1) Definition, Classification, and Clinical Evaluations

Table 1 lists the well-known classification of the periodontal diseases defined by the American Academy of Periodontology in 1999, which was a revisionof the previous classification developed in 1989 [2]. According to the 1989 classification, there were no descriptions of gingival diseases, whereas dental plaque-induced or non-induced gingival

diseases are listed in the 1999 classification. In addition, the term “Adult periodontitis” was replaced with “Chronic periodontitis” based on the epidemiological data and clinical experience, in which this form of periodontitis is also identified in adolescents.

Table 1. Classification of periodontal diseases defined by the American Academy of Periodontology

I. Gingival diseases
A. Dental plaque-induced gingival diseases
B. Non-plaque-induced gingival diseases
II. Chronic Periodontitis
A. Localized
B. Generalized
III. Aggressive periodontitis
A. Localized
B. Generalized
IV Periodontitis as a Manifestation of Systemic Diseases
A. Associated with hematological disorders
B. Associated with genetic disorders
C. Not otherwise specified (NOS)
V. Necrotizing Periodontal Diseases
A. Necrotizing ulcerative gingivitis (NUG)
B. Necrotizing ulcerative periodontitis (NUP)
VI. Abscess of the Periodontium
A. Gingival abscess
B. Periodontal abscess
C. Pericoronal abscess
VII. Periodontitis Associated With Endodontic Lesions
A. Combined periodontic-endodontic lesions
VIII. Developmental or Acquired Deformities and Conditions
A. Localized tooth-related factors that modify or predispose to plaque-induced gingival diseases/periodontitis
B. Mucogingival deformities and conditions around teeth
C. Mucogingival deformities and conditions on edentulous ridges
D. Occlusal trauma

As for the periodontitis identified in young patients, the term “Early-onset periodontitis” was used in the 1989 classification, however, the term was changed to “Aggressive periodontitis” in order to minimize potential problems with age-dependent features of classification. There was another category “Periodontitis associated with systemic diseases” in the 1989 classification, which was changed to “Periodontitis as a manifestation of systemic diseases.” Despite this small change,this category wasbasically retained. In addition, replacement of “Necrotizing ulcerative periodontitis” with “Necrotizing periodontal diseases” and addition of the categories of “Periodontal abscess,” “Periodontic-endodontic lesions” and “Developmental or acquired deformities and conditions” were implemented. These classifications will likely be reviewedin the future based on discussions of updated concepts. Considering periodontal diseases in children, we classify the clinical conditions into gingivitis, chronic periodontitis, invasive periodontitis (localized or generalized), periodontitis associated with systemic disease, and necrotizing periodontitis. In addition, it is clinically useful to designate prepubertal and juvenile periodontitis corresponding to the primary and permanent dentitions, respectively.

In our daily practice, clinical evaluations are performed using the standard parameters of periodontal diseases, *ie.*probing depth, bleeding on probing, pus discharge, tooth mobility, plaque index [3], and gingival index [4]. We generally measure periodontal pocket depths to the nearest millimeter at 6 points around the circumference of each tooth (mesio-, mid-, and disto-buccal; and disto-, mid-, and mesio-lingual) from the gingival margin to the deepest probing point, using a round-ended probe tip 0.4 mm in diameter. Bleeding on probing is scored as follows; (+) immediate bleeding on probing or (-) no bleeding. Tooth mobility is scored as follows; (2) moderate mobility (1~2 mm) in a bucco-lingual direction, and (1) slight mobility (0.2~1 mm) in a bucco-lingual direction, or (0) physiological mobility within 0.2 mm. Pus discharge is scored as follows; (+) spontaneous pus discharge, or (-) no pus discharge.

2) Gingivitis

Gingivitis is defined as localized inflammation of the marginal gingival without resorption of alveolar bone. The affected gingiva shows swelling and redness as well as ready bleeding upon probing or brushing. All of the cases are derived from poor oral hygiene. The basic treatment is mechanical removal of the dental plaque or calculus in combination with professional tooth brushing instructions. Simple gingivitis is the term representing gingivitis initiated by poor oral hygiene conditions (Figure 2). The condition of the inflamed lesion is reversible in most of the cases in children, and removal of the dental plaque or calculus allows the lesionsto return to the normal state. When we encounter the cases of erupting tooth, it is difficult to maintain adequatehygiene conditions in these areas due to the difficulty of cleaning. “Erupting gingivitis,” which represents gingivitis with poor hygiene of the erupting teeth, also belongs to this category (Figure 3).

The incidence of gingivitis in children increases as they grow and reaches its peak at the age of 10-12 years, which we specifically call “Pubertal gingivitis.” This gingivitis is often found in girls with gingival swelling and redness especially at the dental papilla. It is likely that hormonal changesare associated with thisincreased susceptibility to gingival inflammation. Thorough oral hygiene interventions can readilyreverse this condition. On the

other hand, acute necrotizing ulcerative gingivitis (ANUG) is a rare condition in Japan although the incidences in developing countries are reported to be high (Figure 4). The gingival tissues of dental papilla and the gingival margin wereobserved to be red with ulcerative lesions with the morphology of a crater. The ulcerative lesion is gray and covered with a pseudomembrane which is easily removed and even a slight stimulation is known to cause severe pain. At the initial stage, systemic antibiotics rapidly reverses this condition.The lesion should also be differentiated from viral stomatitis. It should be noted that cases with herpetic gingivostomatitis are occasionally encountered in infants and children (Figure 5). The severe inflammation, such as swelling, redness, erosion, is identified with specific foul breath odor. At the initial stages, severe fever is observed, whereas it is generally cured within 2 weeks. However, it takes more time to recover in cases with difficulties in ingesting food due to severe pain in the oral cavity. Antibiotic administration and the adequate availability of water as well as nutrients are very important for healing the lesions.

Figure 2. Two cases of severe gingivitis with a large amount of dental plaque accumulation.

Figure 3. A case of erupting gingivitis at the mandibular left canine (arrow).

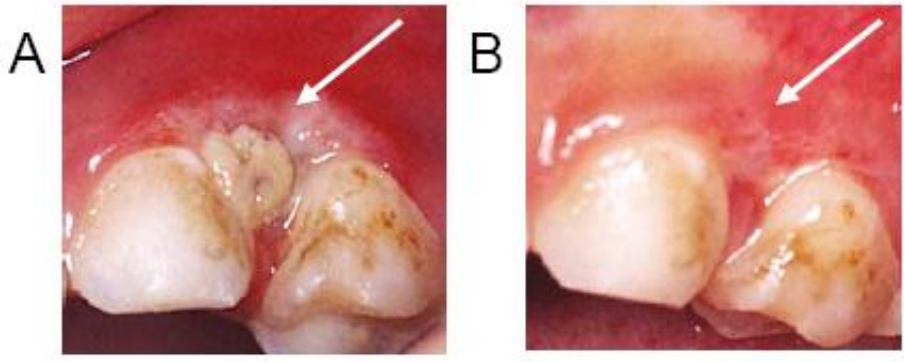

Figure 4. A case diagnosed as acute necrotizing ulcerative gingivitis at first visit (A) and after antibiotic treatment (B).Arrows indicate the affected lesion.

Figure 5. A case diagnosed as acute herpetic gingivostomatitis.

3) Periodontitis

Periodontitis is defined as the disease leading to destructionof periodontal tissues, such as the periodontal ligament, cementum and alveolar bone. Periodontitis in children is generally regarded as an extremely rare finding. In generalized prepubertal periodontitis, the alveolar bone of all teeth are resorbed, with severe redness and swelling due to the intensive inflammation. Although the incidence is extremely low, early exfoliation of primary teeth is prominent. Generalized prepubertal periodontitis is known to be an oral manifestation of leukocytes adhesion deficiency. Antibiotics therapy is carried out to stabilize the lesions in acute inflammation to preventthe lesions progressing gradually leading to the spontaneous exfoliation of the affected teeth. On the other hand, localized prepubertal periodontitis is initiated by sudden pain and mobility of the several limited teeth (Figure 6). The repeated acute attacks develop into progressive alveolar bone loss. The inflammation can be observed only during the period of acute attack and no abnormal findings can be seen in periods without acute inflammation. The incidence is considered to be higher than localized juvenile periodontitis. It should be noted that this category does not include cases of hypophosphatasia associated with problems in the generation of periodontal ligaments. Antibiotic treatment is

carried out to stablize the lesion as with generalized prepubertal periodontitits. In order to preserve the affected teeth as long as possible, thorough oral hygiene instruction and local application of antibiotics are performed.

Localized juvenile periodontitis (LJP) is widely known as the specific form of periodontitis identified in adolescents. The detection frequency of LJP in Japanese adolescents is reported to be 0.06-0.2%. The vertical alveolar bone resorption is found predominantly in the first permanent molars and central incisors (Figure 7) and is identified in females more frequently than in males. Early diagnosis and intervention are required since the speed of the resorption of alveolar bone is very fast. Thorough mechanical teeth cleaning and local application of antibiotics enables control of disease development.

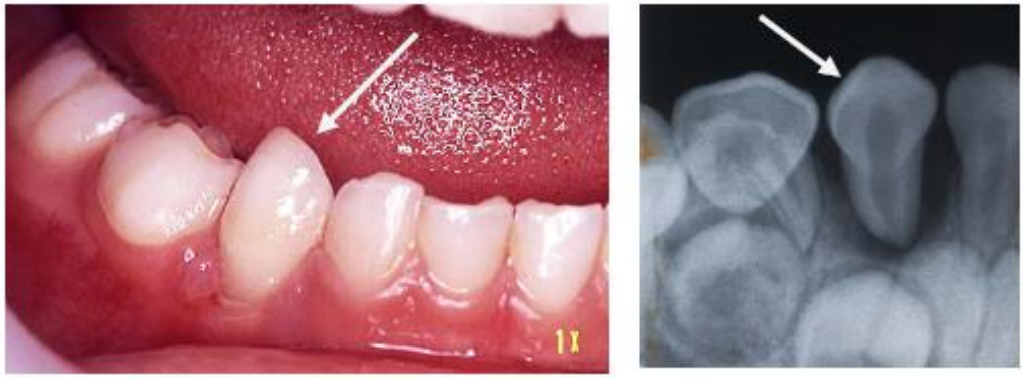

Figure 6. A case diagnosed as localized prepubertal periodontitis. Arrows indicate the vertical resorption of alveolar bone of the affected teeth.

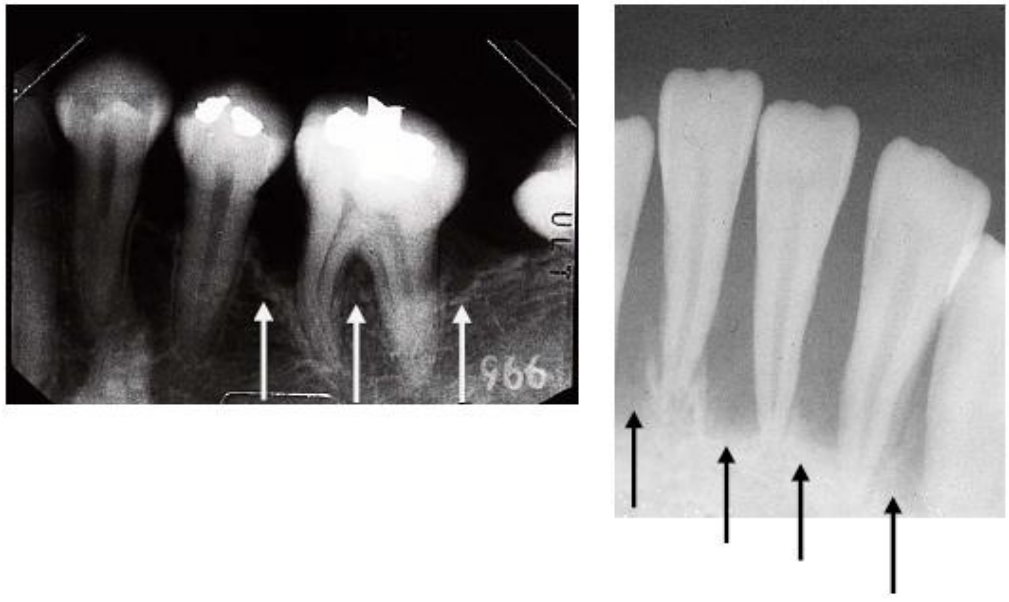

Figure 7. Radiographic features of localized juvenile periodontitis. White and black arrows indicate the resorption of the supportive bone in mandibular first molar and incisors, respectively.

4) Gingival Recession

Gingival recession is occasionally identified at the labial gingiva of mandibular incisor teeth, which is dislocated out of the dental arch due to space limitations. The labial alveolar bone of the teeth is thin due to mechanical forces, such as traumatic occlusion and tooth brushing (Figure 8). In order to solve this problem, the affected teeth should be moved within the dental arch for the former case and instruction for appropriate tooth brushing for the latter case.

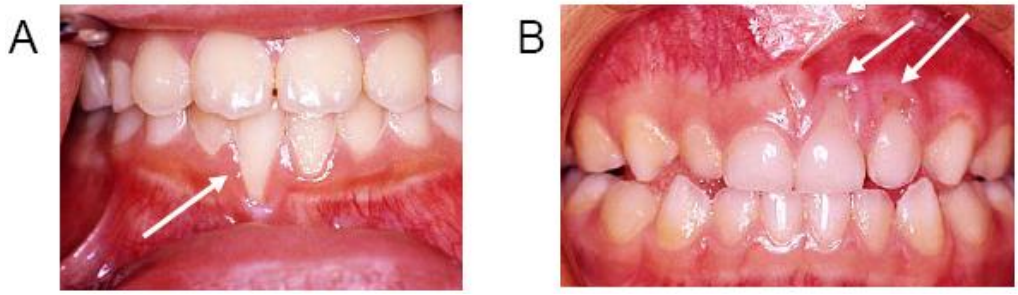

Figure 8. Two cases of gingival recession due to traumatic occlusion (A) and tooth brushing with excessive power (B). Arrows indicate the affected teeth.

5) Gingival Overgrowth

Gingival fibromatosis is a rare overgrowth associated with increased levels of mature collagen and the enlarged gingival tissues are usually normal in color, firm in consistency, painless and occasionally nodular with little inflammation [5]. Gingival fibromatosis causes esthetic and functional problems, such as malposition of teeth, prolonged retention of primary teeth and delayed eruption of permanent successors. In addition, the hyperplastic region produces conditions favorable for accumulation of dental plaque causing secondary inflammatory changes although alveolar bone is not affected. Gingival fibromatosis is known to have hereditary predispositions in some patients. Figure 9 shows a case involving 11-year-old twin brothers, both of which showed typical features of gingival fibromatosis [6]. On the other hand, cases without apparent genetic links are also present, in which specific medication,

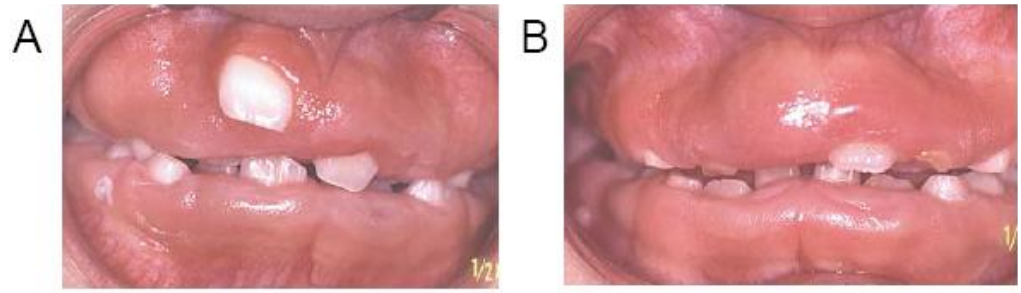

Figure 9. Gingival fibromatosis identified in twin brothers. Intraoral photographs of the older (A) and the younger (B) brothers.

such as phenytoin,commonly used as an antiepileptic, can lead to the onset and development of the lesion (Figure 10). Furthermore, cyclosporine, an immunosuppressant drug, and nifedipine, a calcium channel blocker used as an antihypertensive agent, are also known to cause similar gingival overgrowth [7]. Phenytoin is known to stimulate responsive subpopulations of gingival fibroblasts to accumulate extracellular matrix components, resulting in gingival overgrowth [8], whereas several studies have found a relationship between the quantity of accumulated dental plaque and phenytoin-induced gingival overgrowth [9-11]. It was also recently indicated that dental plaque accumulation is the most important determinant of phenytoin-induced gingival overgrowth [12]. Therefore, it is now believed that enhanced matrix synthesis by fibroblasts responsive to phenytoin can be triggered or enhanced by chronic inflammation due to dental plaque [13]. In general, professional teeth cleaning and tooth brushing instruction are performed and gingivectomy is carried out for severe cases although recurrence of the lesion is often observed.

A 10-year-old girl was referred to our clinic for consultation due to the swollen gingiva in her incisor regions that caused esthetic problems (Figure 11) [14]. Intraoral examinations showed severe generalized gingival overgrowth involving both maxillary and mandibular teeth, which covered almost half of the crown. She had no medical disorders and none of the family membersexhibited any gingival problems. Gingivectomy was carried out under local anesthesia, which solved her esthetic problems. Histopathological analyses showed the typical appearance of gingival fibromatosis. There were no recurrences of the lesion reported in this case. However, it is possible to speculate that poor oral hygiene can lead to the recurrence of overgrowth, which should be periodically monitored.

Figure 10. Gingival fibromatosis identified in subject with specific treatment with the antiepileptic phenytoin.

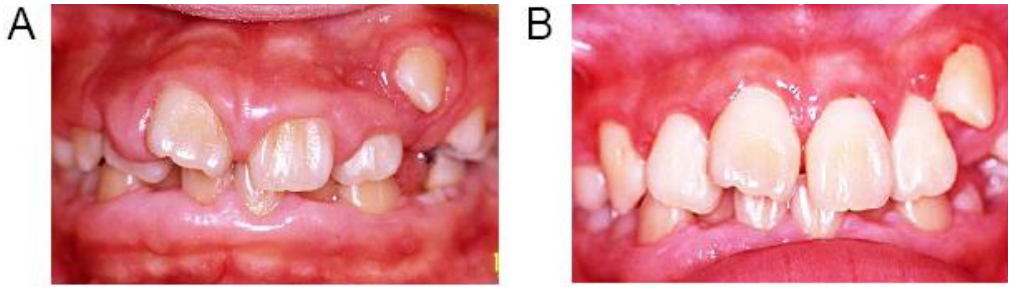

Figure 11. Preoperative (A) and postoperative (B) photographs in a case of gingival fibromatosis.

6) Acute Periodontitis

Acute periodontitis is not listed in the classification now used in the field of periodontology. However, cases of rapid loss of gingival attachment and alveolar bone resorption development in a couple of days are described in the oral pathology literature. Appropriate interventions enable recovery to healthy periodontal conditions for several months. The initiation of these conditions is considered to be the result of infection bypyogenic bacteria at the sites of small injuries present in the gingival sulcus. Although rarely encountered, irrigation of the gingival pocket and systemic antibiotictherapy generally suppress acute inflammation within a week.

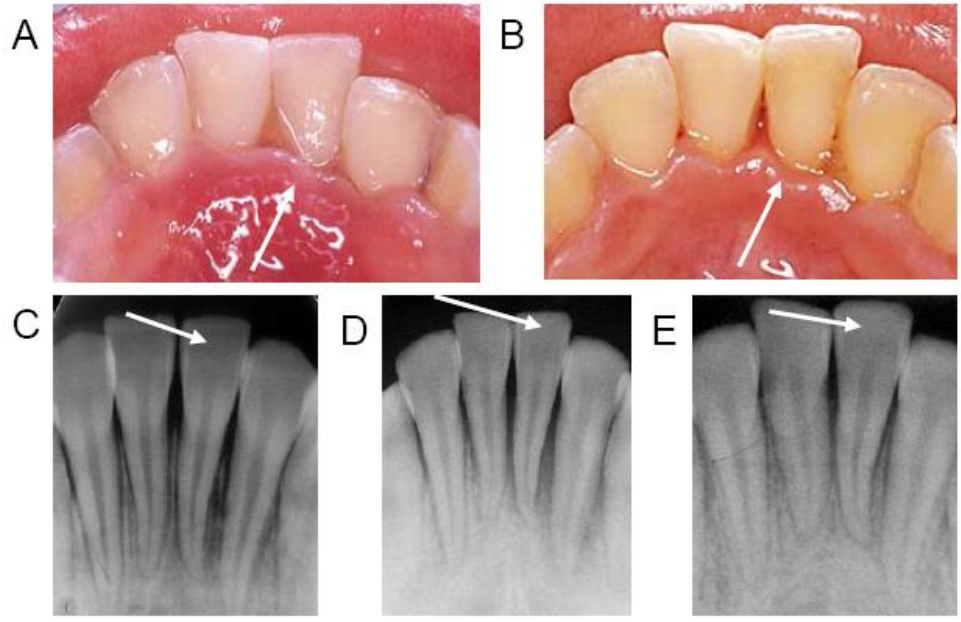

Figure 12. A case of "acute periodontitis." Intraoral photographs taken at first examination (A) and at the time when the lesion became stabilized (B). Periapical radiographs taken at the first examination (C), 2 weeks (D) as well as 4 months (E) after the first examination. Arrows indicate the affected tooth.

A 10-year-old Japanese girl came to our hospital with the chief complaint of severe tooth mobility in her lower permanent incisors (Figure 12) [15]. The incisors were shown to have severe alveolar bone loss and periodontal pocket depths exceeding 7 mm. Periodontal treatment consisting of mechanical debridement and antibiotic medication resulted in a significant improvement of the clinical parameters. Three months after the first examination, periapical radiographs showed refilling of the alveolar bone in the affected tooth. It is of interest that microbiological examinations at the first visit did not identify any typical periodontitis-related pathogens, whereas several periodontitis-associated species were identified in the examinations held after the healing of the lesions.

Orthodontic bands could also be one of the possible initiators of acute periodontitis. An 11-year-old boy was referred to our clinic for treatment of gingival swelling and severe occlusal pain around the mandibular left permanent molar (Figure 13, Table 2) [16]. Intraoral examinations showed that gingival swelling with apparent redness around the affected tooth.

The maximum periodontal pockets depth was 9 mm and the affected tooth showed severe mobility. According to the orthodontist, the orthodontic band was removed just before visiting our clinic. Periapical radiograph showed alveolar bone loss on the distal side. Irrigation of the marginal gingiva with systemic antibiotics was performed. Twelve days later, inflammation of the affected gingiva had diminished andthe maximum periodontal pocket was reduced to 6 mm. Three months later, bleeding on probing had stopped and the maximum periodontal pocket was reduced to 3 mm. Interestingly, there was no typical periodontitis-related species identified at the first examination, whereas some of the species were detected after the lesion recovered.

Figure 13. A case of "acute periodontitis" caused by orthodontic band. Intraoral photograph of the affected tooth taken at 1st visit (A) and 5th visit (98 days after first visit) (B).

Table 2.Transitional changes of the periodontal condition in acute periodontitis associated with orthodontic band

	Days after the first visit					
	0	5	12	30	98	147
Periodontal pocket depth (mm)	9	7	6	4	3	2
Bleeding on probing	+	+	+	+	-	-
Gingival index	2	1	1	1	1	1
Plaque index	0	1	1	2	1	1
Tooth mobility	3+	+	+	-	-	-
Pus exudate	-	-	-	-	-	-

7) Accidentally-Induced Periodontitis

Accidentally-induced periodontitis is unique for infants and younger children in which the materials fitted to the teeth, such as small plastic tubes, are accidentally inserted into the tooth crown and cause attachment loss (Figure 14). In our clinic, only 4 cases have been encountered over a period of approximately 40 years, all of which were accidentally induced by the insertion of plastic tubes into the lower primary central incisor region. In 3 of those, a single plastic tube had been inserted into the left lower primary central incisor area, whereas 2

tubes were found simultaneously in the dental cervix of the lower primary central incisors. Figure 15 shows the summary of the case induced by 2 tubes [17].

Figure 14. A case of accidentally-induced periodontitis. Arrows indicate the affected teeth.

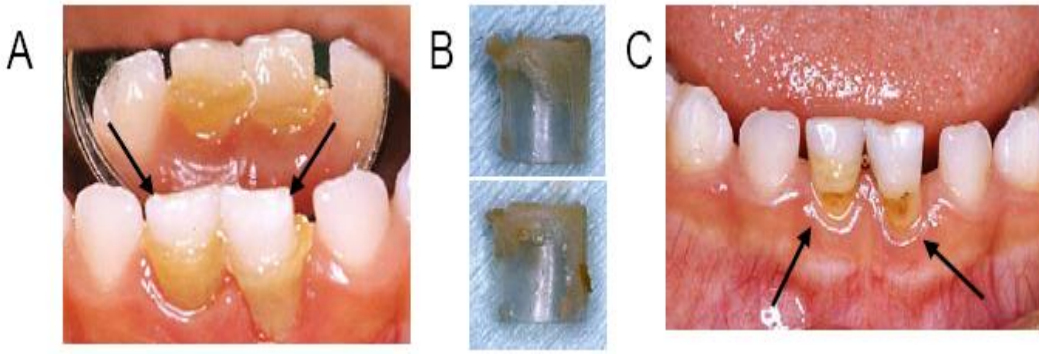

Figure 15. A case of accidentally-induced periodontitis. Intraoral photograph of 1st visit (A), plastic tubes removed from the affected teeth (B), and intraoral photograph taken one year after the removal of the tubes (C). Arrows indicate the affected teeth.

A 4-year-old boy was referred with the chief complaint of swelling around his lower primary incisors. Clinical examinations revealed inflamed gingival tissue around the lower primary central incisors and severe mobility of these teeth. Small transparent plastic tubes were found in the dental cervix of the lower primary central incisors, which were likely accidentally inserted during play. A periapical radiograph revealed diffuse alveolar bone loss between the lower primary central incisors. Irrigation of the affected teeth was performed. Table 3 summarizes the transitional changes in his periodontalhealth. Three months after the first visit, an examination revealed recovery of gingival attachment, and a periapical radiograph showed that the alveolar bone defects between the lower central incisors were being repaired. However, the periodontal condition of the affected teeth could not be restored to their original status. It was concluded that the type of periodontitis caused by such an incident is not progressive, unlike other periodontal diseases such as prepubertal and juvenile periodontitis.

Table 3.Transitional changes of the periodontal condition in acute periodontitis associated with plastic tubes insertion

	Months after the first visit					
	0	0.5	3	6	12	24
Periodontal pocket depth (mm)	5	4	2	2	2	2
Bleeding on probing	+	-	-	-	-	-
Gingival index	2	1	0	0	0	0
Plaque index	1	1	0	0	0	1
Tooth mobility	2+	+	-	-	-	-
Pus exudate	-	-	-	-	-	-

8) Periodontitis Associated with Systemic Diseases

In spite of the extremely low frequency of periodontitis in systemically healthy children, periodontitis in children is identified in certain types of systemic diseases mainly due to the impairment of the host immune response. It is well known that patients with neutropenia, Chédiak-Higashi syndrome, Papillon-Lefèvre syndrome, Down's syndrome, diabetes mellitus, hypophosphatasia, Histiocytosis syndrome, Ehlers-Danlos syndrome, and acquired immunodeficiency syndrome, are prone to develop periodontitis [18].

Hypophosphatasia is generally known as one of the systemic diseases associated with periodontitis. It is an inheritable disorder characterized by hypomineralization of bone associated with the impaired activity of tissue-nonspecific alkaline phosphatase, and the disease is highly variable in clinical expression, ranging from an almost total lack of skeletal formation to the premature loss of the permanent anterior teeth [19]. There are 5 subtypes based on the age of onset and clinical features; perinatal, infantile, childhood and adult types, and odontohypophosphatasia, in which only the teeth are affected. The common clinical signs are premature exfoliation of primary teeth, which is thought to be caused by adefect in cementum formation althoughsome reports have noted that accumulation of bacteria accelerates the exfoliation [20, 21]. Thus, initiation of periodontitis in patients with hypophosphatasia involves periodontal pocket formation without a clear inflammatory process and is caused by cementum impairment due to a low alkaline phosphatase concentration. It is difficult to prevent the early exfoliation of the primary incisors although only a limited number of teeth, mainly mandibular anterior teeth, are affected. In addition, it is extremely rare to observe cases with early exfoliation of the permanent teeth. Local antibioticirrigation and professional tooth brushing instruction may lead to modulation of the exfoliation period.

Figure 16 shows a case of twin brothers complicated with hypophosphatasia [22].At 3Y4M, an intraoral examination of the elder brother showed that the mandibular left primary lateral incisor was missing and exposure of the root of the mandibular right primary canine was prominent. In contrast, there were no specific problems in the younger brotheralthough one tooth was missing due to a previous traumatic injury. The elder brother was diagnosed with periodontitis and professional tooth cleaning was performed, while brushing instructions

were given to the patient and his parents to prevent progression to tooth exfoliation. Periodical examinations were carried out and 2 additional teeth were found exfoliated in the elder brother. At 5Y3M, there were 15 teeth identified in the elder brother, while 19 teeth were identified in the younger brother. Although cases with twin brothers are considered to result from genetic influences, their teeth phenotypes were totally distinct.

Figure 16. Cases of hypophosphatasia identified in twin brothers. Intraoral photographs taken at 3Y4M and 5Y3M for older (A and C) and younger (B and D) brothers, respectively.

9) Peridontitis Associated with Anatomical Anomalies

A tooth with a radicular gingival groove is considered to be susceptible to periodontitis due to the weak binding of periodontal ligamentsto the root surface. Although it is not common that anatomical problems have effects on the development of periodontitis, the radicular-gingival groove is an anatomical anomaly of the teethwith a reported prevalence of 2-4%, with the maxillary lateral incisors regarded as the area with the most frequent occurrence [23-25]. Such a groove is sometimes found as a radiolucent line in radiographic examinations and its main feature has been described as a "parapulpal line," which is similar to the line produced by a vertical tooth fracture [26-30].The chief complaint regarding the lesion caused by the groove is gingival swelling and pain, and root canal treatment or a flap operation is typically selected as general treatment modalities for severe cases. Nevertheless, the prognosisfor the lesions is considered to be poor [26-32].

An 11Y5M female came to our clinic with a chief complaint of severe gingival inflammation in the mandibular left lateral incisor (Figure 17). The periapical radiograph showed a parapulpal line and the lesion was estimated to be derived from the radicular-gingival groove [33]. A gingivectomy was carried out, followed by local irrigation and thorough instructions regarding tooth brushing. After a long interval between examinations, she returned to our clinic at the age of 18Y4M and reported repeated slight swelling that had occurred for several years, although without severe signs or symptoms. We rationalized that the lesion is susceptible to inflammation due to her anatomical anomaly, however, careful oral hygiene possibly stabilized the lesion. According to previous reports regarding cases with radicular-gingival grooves, the subject ages range from 12 to 45 years old and the maximum periodontal pocket depths were between 6 and 9 mm, which areregarded as severe conditions

[26-32]. Prognoses are considered to be poor and extraction of the affected teeth was reported in most of the cases in a range of 6 months to 3 years, while no significant recurrent signs or symptoms were observed for 1.5 to 3 years in several of the cases. Thus, periodical observation is important once we identify teeth with radicular-gingival grooves in order to intervene in the onset and development of periodontitis.

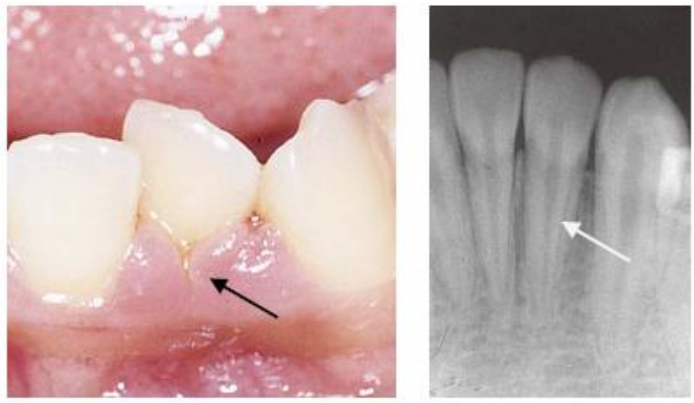

Figure 17. A case of periodontitis associated with radicular-gingival groove. Black and white arrows indicate the affected gingival lesion and parapulpal line, respectively.

3. Molecular Biological Analyses

1) Detection of Periodontal Bacteria

The bacterial flora in human dental plaque is diverse and complex, consisting of more than 700 bacterial species, whereas a small subset of the species is considered to be the primary agents of periodontal diseases [34-38]. The periodontal pathogens generally belong to the obligate anaerobe group, and isolation of these species is technically very demanding and time-consuming. On the other hand, the recent development of molecular biological techniques enables the analysis of the periodontitis-related species without bacterial isolation. The PCR technique using the bacterial DNA extracted from the specimens can identify the bacteria with species-specific sets of primers [39-43], whereas the disadvantage of this approach is that only selected species can be analyzed. In contrast, broad-range PCR and sequence methods can demonstrate the bacterial profiles in the specimens since the primers are designed for the common region of the 16S rRNA sequences for eubacterial species [44, 45]. The advantage of this method is that it can identify any species registered in the genomic databasesbut it takes more time and is relatively expensive compared to the conventional PCR method. In addition, a quantitative PCR method can be used to determine the bacterial levels in the specimens [46]. Although it would be valuable to determine the numbers of each species in the specimens, it would be relatively expensive to do so at present with large numbers of specimens in large-scale clinical studies.

It is also important to decide whether saliva or dental plaque specimens are to be analyzed when using these techniques. Whole saliva specimens generally reflect the entire bacterial profiles in the oral cavity, whereas the profiles of the dental plaque specimens are

limited to those of the collected area. Thus, we must carefully consider which specimens match the purpose of studies prior to their initiation. In addition, the timing of collection of the specimens is also important. We generally collect the specimens for the initial stage at the second visit to our clinic, prior to giving detailed tooth brushing instructions, which enable us to determine the natural bacterial profiles of each subject. The procedures for extraction of the bacterial DNA are as follows. The subgingival dental plaque specimens are collected in sterile saline, which are centrifuged at 15000 rpm for 5 min to pellet the bacterial cells. Bacterial genomic DNA is extracted from each pellet using a DNA isolation kit (Puregene, Gentra Systems, Minneapolis, MN, USA). As for the saliva samples, expectorated whole saliva collected from each patient is mixed with Chelex 100 (Bio-Rad Laboratories, Hercules, CA, USA), which is then incubated at 56°C for 30 min, followed by boiling at 100°C for 10 min. Each sample is then centrifuged at 15000 rpm for 20 min and the supernatants used as templates for PCR assays.

Table 4. Primer list for detection of 10 periodontitis-related species

Species	Sequence (5'to 3')	Amplification size (bp)	References
Positive control	AGA GTT TGA TCM TGG CTC AG CTG CTG CSY CCC GTA G	315	[43]
Pg	TGT AGA TGA CTG ATG GTG AAA ACC ACG TCA TCC CCA CCT TCC TC	197	[41]
Td	AAG GCG GTA GAG CCG CCG CTC A AGC CGC TGT CGA AAA GCC CA	311	[42]
Tf	GCG TAT GTA ACC TGC CCG CA TCG TTC AGT GTC AGT TAT ACC T	641	[39]
Co	AGA GTT TGA TCC TGG CTC AG GAT GCC GTC CCT ATA TAC CAT TAG G	185	[40]
Cs	AGA GTT TGA TCC TGG CTC AG GAT GCC GTC CCT ATA TAC GGG G	185	[40]
Pi	TTT GTT GGG GAG TAA AGC GGG TCA ACA TCT CTG TAT CCT GCG T	575	[39]
Pn	ATG AAA CAA AGG TTT TCC GGT AAG CCC ACG TCT CTG TGG GCT GCG A	804	[39]
Aa	AGA GTT TGA TCC TGG CTC AG CAC TTA AAG GTC CGC CTA CGT GCC	593	[40]
Cr	TTT CGG AGC GTA AAC TCC TTT TC TTT CTG CAA GCA GAC ACT CTT	598	[39]
Ec	CTA ATA CCG CAT ACG TCC TAA G CTA CTA AGC AAT CAA GTT GCC C	688	[39]

Various bacterial species have been reported to be related to periodontitis, among which we recently focused on 10 species, including *Porphyromonas gingivalis* (Pg), *Tannerella forsythia* (formerly *Tannerella forsythensis*) (Tf), *Prevotella intermedia* (Pi), *Prevotella nigrescens* (Pn), *Campylobacter rectus* (Cr), *Eikenella corrodens* (Ec), *Aggregatibacter*

(formerly *Actinobacillus*) *actinomycetemcomitans* (Aa), *Capnocytophaga ochracea* (Co), *Capnocytophaga sputigena* (Cs) and *Treponema denticola* (Td) [47-49], based on previous reports showing that their distributions in periodontitis patients were significantly different from those in periodontally healthy subjects [50-55]. Table 4 lists the primer sets specific for each species used in our studies, all of which were checked for specificity as well as sensitivityand was reported to range from 10-100 cells in the original studies [39-43]. Figure 18 shows an example of the results for the detection of 10 periodontal bacterial species by PCR with species-specific sets of primers. There were two species identified in the saliva specimen of subject A, whereas 6 species were detected in the specimen taken from subject B.

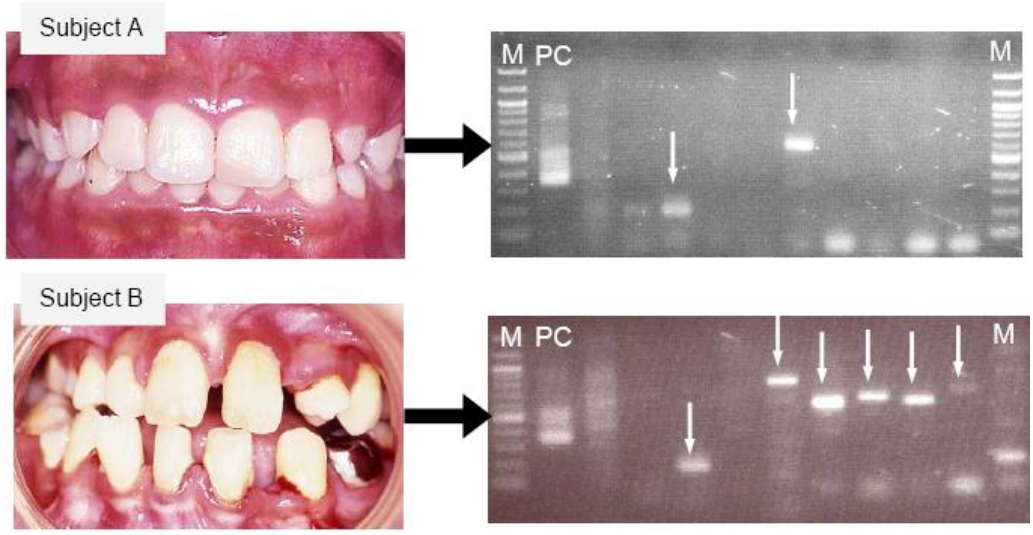

Figure 18. Detection of periodontal bacterial species in oral specimens by PCR methods. The left pictures show the two representative cases (subjects A and B) and the right images are stained electrophoresis gels for identification of the species in the specimens taken from each case. “M” and “PC” indicate molecular marker and positive control, respectively, and arrows indicate the positive reactions.

2) Distribution of Periodontal Bacteria in Children

There have been few reports describing the results of the distribution of periodontal bacterial species in children and adolescents when we initiated this study. It was generally known that children with periodontitis are rarely encountered, which might be one of the reasons for the few studies analyzing their distribution. Therefore, we decided to analyze the distribution of the 10 selected bacterial species in the dental plaqueand saliva specimens in children and adolescents who came to our clinic. Dental plaque specimens were collected from the buccal-mesial sulcus of the first molar or second primary molar in the right upper quadrant of 119 systemically healthy children (56 boys and 63 girls) aged 2 to 13 years old, who showed negligible periodontal inflammation, and their whole expectorated saliva specimens were also collected [47, 48]. The total numbers of dental plaque and saliva

specimens collected from these subjects were 300 and 208, respectively. Those subjects had received no antibiotic medication for at least 3 months prior to each specimen collection. PCR

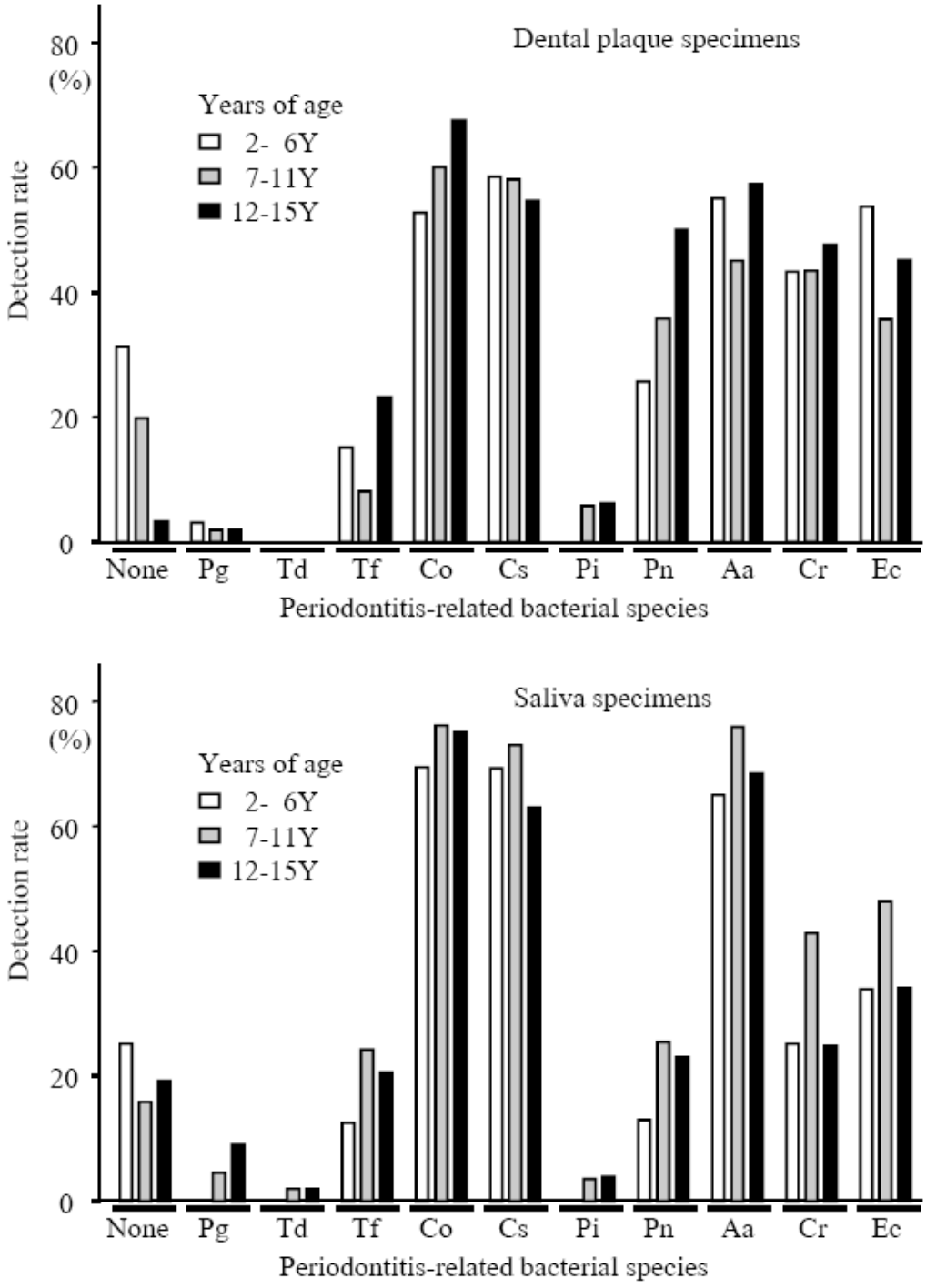

Figure 19. Distribution of periodontal bacterial species in dental plaque and saliva specimens taken from periodontally healthy children aged 2-15 years old.

detection using 10 species-specific primer sets showed that approximately 15% of the subjects were negative for any of those 10 species. The total numbers of the detected species increased gradually with age until 5 years old and then reached a plateau after the mixed dentition period. The detection rates for most of the species in saliva specimens were significantly higher than those in dental plaque specimens. *C. rectus*, *E. corrodens*, *A. actinomycetemcomitans*, *C. ochracea*, and *C. sputigena* were found in approximately 50% of

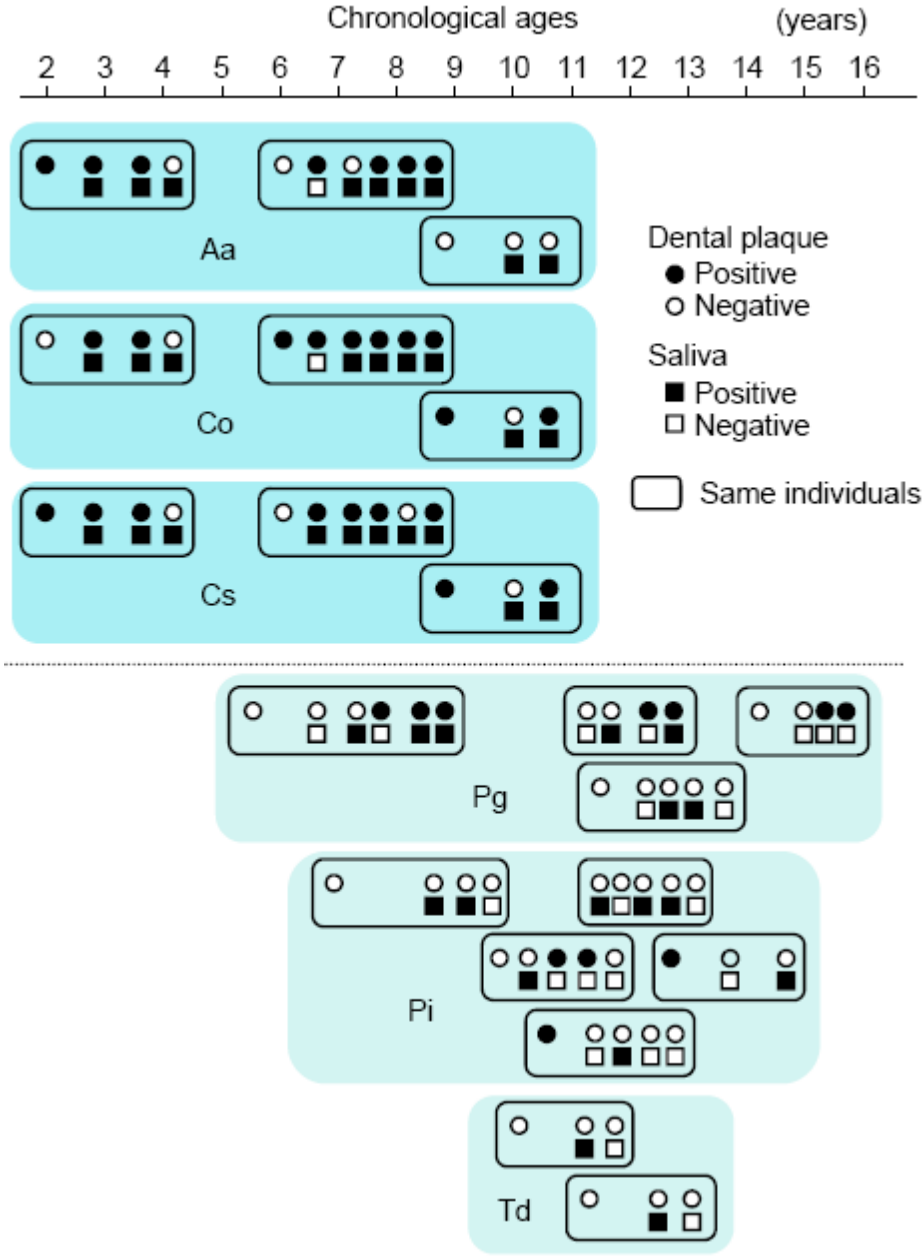

Figure 20. Longitudinal monitoring of periodontal bacterial species in periodontally healthy children for determination of whether they are transient or not.

all age groups, while *T. forsythia* and *P. intermedia* were detected less frequently, and *P. gingivalis* and *T. denticola* were rarely identified (Figure 19). These results suggest that the colonization of many periodontal bacterial species occurs quite early in childhood without clinical signs of periodontal disease. In contrast, there are several species exhibiting poor colonization, such as *P. gingivalis* and *T. denticola*.

Next, in order to determine which species are inhabitants or are transient, the subjects whose specimens were collected more than twice for more than 2 years were analyzed. Figure 20 shows one of the representative results. *C. ochracea*, *C. sputigena*, *A. actinomycetemcomitans*, *P. nigrescens*, *C. rectus*, and *E. corrodens* were frequently detected in multiple specimens taken at different times, suggesting that these are the common members of the oral flora of periodontally healthy children. In contrast, *P. gingivalis*, *T. denticola*, and *P. intermedia* were rarely detected which indicates that these species should be regarded as transient species in children. These results suggest that there exist some species with early colonization and that others are just transient species in children.

3) Longitudinal Studies of Periodontitis-Assocated Bacteria

It is of interest to determine the distribution of the periodontal species in subjects over time. The occurrence of periodontal bacterial species in 192 systemically healthy subjects (89 male and 103 female, 2–16 years old) was analyzed in 1999-2000, among which 26 subjects continued to attend annual recall examinations until 2006–2007 [56]. Thus, a total of 26 children and adolescents from whom dental plaque and saliva specimens were obtained during both the 1st (1999-2000) and 2nd (2006-2007) periods, were analyzed. The periodontal condition of most of the subjects in the 2nd period wasrelatively good, which reflected their continuous participation in our recall system, in which not only periodical examinations were performed but also professional tooth cleaning as well as brushing instructions were provided. On the other hand, there were several subjects with periodontal pocket depths of 4-5 mm without attachment loss. The PCR method specified the presence of 10 periodontal bacterial species, which indicated a positive correlation of periodontal pocket depth with the total number of detected species. *P. gingivalis*, *T. denticola*, and *T. forsythia*comprise the "red complex" (RC) group of species, a prototypical polybacterial pathogenic consortium active in periodontitis [57, 58]. RC species are also known to be correlated with gingival inflammation based on studies of Japanese teenagers [59, 60]. We further analyzed the bacterial profiles in the first and second collections focusing on the presence of RC species [56].

Subjects with RC species in saliva specimens obtained during the 2nd collection possessed a significantly higher number of total bacterial species than those without these organisms (Figure 21). The main reason for this result may be because *P. nigrescens*, *C. rectus*, and *E. corrodens* were detected at significantly higher rates in the subjects with RC species. The detection rate of the RC species in the 2nd collection period specimens was significantly greater in subjects who had 2 or more species detected in specimens taken during the 1st collection compared with the other subjects. In addition, the total number of bacterial species in each of the saliva specimens positive for RC species was greater than 4. Furthermore, retrospective analyses of specimens from subjects with RC species in 1999-2000 showed that those subjects possessed a significantly greater number of total species than

the subjects without RC species. When all specimens obtained at the 1st collection were divided into 2 categories based on the number of total bacterial species, high (2 or more) and low (1 or 0), the detection rate of the RC species at the 2nd collection was significantly greater in the high group (Odds Ratio; 17.5, 95% confidence interval; 1.2-250.4). These results indicate that those subjects who harbor the RC species may be at possible risk for colonization by high levels of periodontal bacterial species during adolescence and early adulthood.

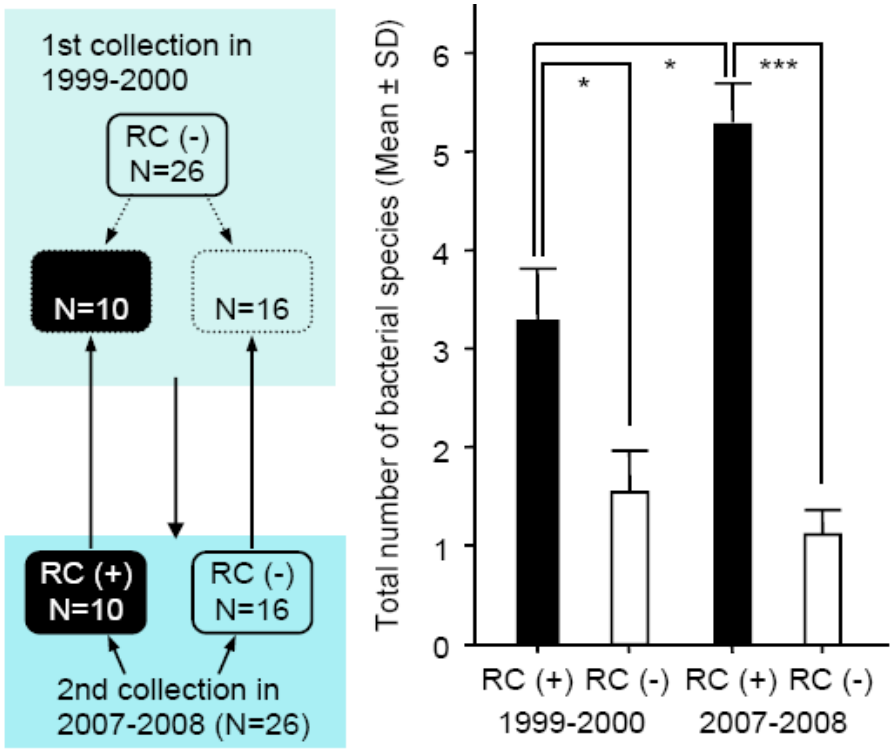

Figure 21. Comparison of the total number of detected species between groups of subjects with and without the red complex (RC) species in 2007-2008. The total numbers of the species of 2007-2008 and 1999-2000. (*P<0.05, ***P<0.001).

4) Multiplicity of Species Detected

Interactions between bacterial species that reside in the biofilms are reported to influence the composition of the communities [61].These interspecies interactions are known to play an essential role in balancing competition and coexistence. Furthermore, synergistic interactions may stimulate the growth or survival of one or more of the residents [62, 63]. In order to determine which species are present simultaneously in the oral cavity, we analyzed the saliva specimens from 113 children (61 boys and 52 girls) aged 2-12 years old [64], which revealed 9 combinations of the species simultaneously detected (Figure 22). It should be noted that *C. rectus* and *E. corrodens* tended to be detected simultaneously with the RC species. Another study analyzing 74 children (39 boys and 35 girls) aged 2-13 years old demonstrated that the

presence of *C. rectus* was correlated withat least one of the RC species (Odds Ratio; 10.4, 95% confidence interval; 1.8-59.6) [65].

The prevalence of *T. forsythia* and *C. rectus* in children was low when compared with the other species, while statistical analysis revealed that *T. forsythia* and *C. rectus* tended to be detected simultaneously in the children [64]. It is of interest that the number of detected species in the children with *T. forsythia* and/or *C. rectus* was 3.71 ± 1.71, which was significantly higher than that for those without either of those species (1.15 ± 0.92). In addition, two children with *P. gingivalis* were found to possess both *T. forsythia* and *C. rectus*. The other study analyzing 107 Japanese children aged 8-11 years old and adolescents aged 15 years old showed that *P. gingivalis* and *T. forsythia*, as well as *T. forsythia* and *C. rectus* were markersfor increased risk of developing periodontitis [60]. Taken together, it is possible to speculate that detectionof these species in Japanese children and adolescents might indicate that these subjects are at high risk of developing periodontitis in the future. It will be of interest to determine whether this hypothesisis applicable to other ethnic groups as well.

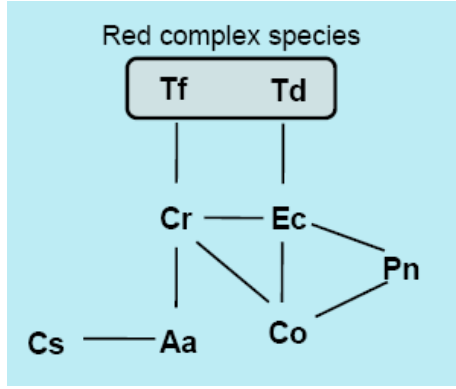

Figure 22. Combination of the periodontal bacterial species simultaneously detected in the same specimens. Lines indicate the combinations of the species simultaneously identified in the same specimens.

5) Mother-To-Child Transmission

It is generally believed that *Streptococcus mutans*, a pathogen of dental caries, can be transmitted from mothers to their children, and our molecular biological analyses showed that the transmission rates of *S. mutans* in Japanese children aged 2-10 years old was approximately 70% [66]. Another study analyzing Japanese children aged 0-11 years old showed that the transmission rate was 76.5% [67]. Thus, *S. mutans* is frequently transmitted early from mothers to their children, which reflects an environment involving close contact between children with their mothers. As for the periodontal bacterial species, intrafamilial transmission of *P. gingivalis*, *A. actinomycetemcomitans*, *P. intermedia*, and *P. nigrescens*, has been studied using various methods, all of which required isolation of each strain [68-71]. The difficulty in carrying out such approaches is the major reason why there have been only a limited number of studies regarding mother-child transmission of periodontal bacterial species. However, the acquisition of periodontal organisms as the initial important step in the development of periodontal diseases in children and adolescents has been discussed, with a

focus on intrafamilial transmission [55]. On the other hand, PCR methods using the bacterial DNA extracted from the oral specimens can readily identify organisms in children and their mothers. Thus, we decided to compare the distribution of oral bacteria in children and their mothers in order to understand which periodontal bacterial species are transmitted from mothers to their children.

Saliva specimens were collected from 113 pairs of children (61 boys and 52 girls) aged 2-12 years and their mothers aged 24-49 years [72]. PCR detectionidentifies the species estimated to be involved in mother-to-child transmission based on the results of the detection rates in both groups. *T. denticola* was detected most frequently (83%) in children whose mothers possessed the same pathogen and was shown to be significantly higher than that in children whose mothers did not harbor the spirochete.In addition to *T. denticola*,*C. sputigena*, *A. actinomycetemcomitans*, and *E. corrodens* showed this same tendency for which the odds ratiosare shown in Figure 23. These resultssuggest that a correlation between the presence of periodontal bacteria in children and their mothers, and the presence of RC bacteria in children was shown to be highly associated with the prevalence in their mothers. As for the other species, the detection rates for*C. sputigena* and *A. actinomycetemcomitans* were relatively high in both mothers and children. Thus, the presence of these species in the mother-child pairs might have been coincidental and were not statistically analyzed. In addition, the numbers of children with *P. gingivalis* were too low in the present study to estimate transmission from mother to child. However, another study demonstrated a tendency for detection of *P. gingivalis* in children whose mothers were also positive for this organism[64]. In addition, the genotypes of *P. gingivalis* fimbriae genesfor mother-child pairs were shown to be consistent which led us to speculate that *P. gingivalis* is also one of the species transmitted from mothers to children. An additional study analyzing 56 Japanese children and adolescents aged 1-15 years and their parents suggested that *P. intermedia*, *P. nigrescens*, and *T. forsythia* were transmitted in an intrafamilial manner [73].

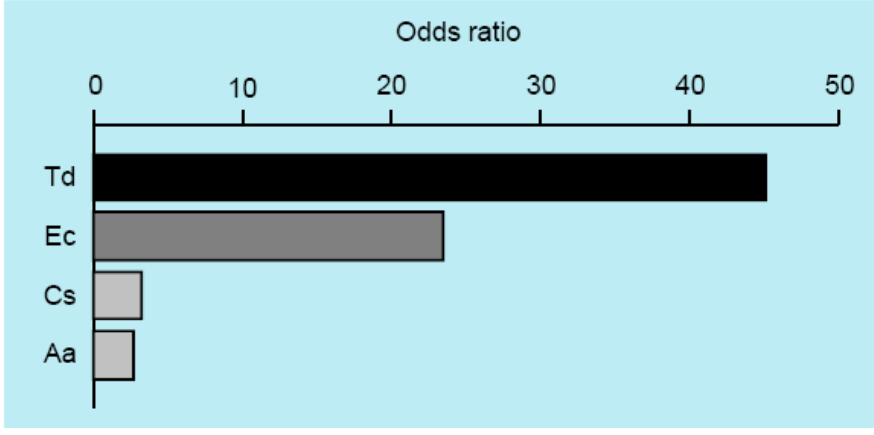

Figure 23. Odds ratios of the species with estimated mother-to-child transmission based on the distribution of the species in children and their mothers.

As described above, RC species are reportedly associated with gingival inflammation based on studies of Japanese teenagers [59, 60]. In our study, approximately 40% of the saliva specimens taken from mothers were shown to possess RC species and their total number of

periodontal bacteria was significantly higher than in those without RC species [64]. The detection rate of the RC species in children whose mothers possessed the same species was shown to be significantly higher than in those whose mothers did not (odds ratio 7.4). A similar study conducted in the United States demonstrated that children whose parents were colonized by RC species were 9.8 times more likely to be colonized by the same species [74]. In addition, the total number of bacterial species in children whose mothers possessed the RC species was shown to be significantly greater than in those whose mothers did not. Taken together, the present data suggested that mothers should be careful not only regarding the current periodontal condition of their children but also their own periodontal health in order to prevent the early subsequent onset of periodontitis in their children.

6) *Porphyromonas gingivalis* Fimbriae

P. gingivalis is considered to be one of the most important pathogens in periodontal disease. This pathogen expresses fimbriae, filamentous appendages on the bacterial surface, which are thought to play a significant role in colonization and invasion of periodontal tissues [75]. FimA, a subunit protein of the major fimbriae, is encoded by the *fimA* gene which is classified into six variants (types I through V and Ib) [76-78]. Table 5 lists the primer sets for identification of *fimA* genotypes in the clinical specimens. A majority of the *P. gingivalis* organisms isolated from marginal periodontitis patients are reported to belong to type II, followed by type IV, while type I tends to be prevalent in periodontally healthy adults [79]. In fact, several other studies conducted in different countries also support our findings that type II *fimA* organisms are strongly correlated with the development of periodontitis [80-82]. These findings suggest the existence of disease-associated and non-associated *P. gingivalis*.

Table 5. Primer list for determination of *fimA* genotypes of *P. gingivalis*

fimA genotype	Sequence (5'to 3')	Amplification size (bp)	References
Type I	CTG TGT GTT TAT GGC AAA CTT C AAC CCC GCT CCC TGT ATT CCG A	392	[76]
Type II	ACA ACT ATA CTT ATG ACA ATG G AAC CCC GCT CCC TGT ATT CCG A	257	[76]
Type III	ATT ACA CCT ACA CAG GTG AGG C AAC CCC GCT CCC TGT ATT CCG A	247	[76]
Type IV	CTA TTC AGG TGC ATA TAC CCA A AAC CCC GCT CCC TGT ATT CCG A	251	[76]
Type V	AAC AAC AGT CTC CTT GAC AGT G TAT TGG GGG TCG AAC GTT ACT GTC	462	[77]
Type Ib	CAG CAG AGC CAA AAA CAA TCG TGT CAG ATA ATT ATT AGC GTC TGC	294	[78]

Further, 70% of periodontally healthy adults with *P. gingivalis* were found to carry type I, the remaining carried type V fimbriae and there were a few subjects positive for types II, IV

and Ib. Figure 24 illustrates the odds ratios showing the relationship between *P. gingivalis fimA* genotypes and development of periodontitis in the subjects. Type II was the highest, followed by types IV and Ib, all of which are regarded as highly virulent groups, whereas types I, III and V are considered to be low virulence groups. In addition, the results of *in vitro* analyses using gingival epithelial cells as well as a mouse model of abscess formation supported this suggestion [83, 84]. Specifically, it was interesting to observe that a mutant with a substitution of the type I *fimA* gene with that of type II showed enhanced bacterial adhesion/invasion to epithelial cells, whereas that with substitution of type II *fimA*with type I resulted in diminished adhesion/invasion [85]. These results suggest that it is possible to estimate the risk of subjects for periodontitis by analyzing the types of *P. gingivalis fimA* genes in oral specimens.

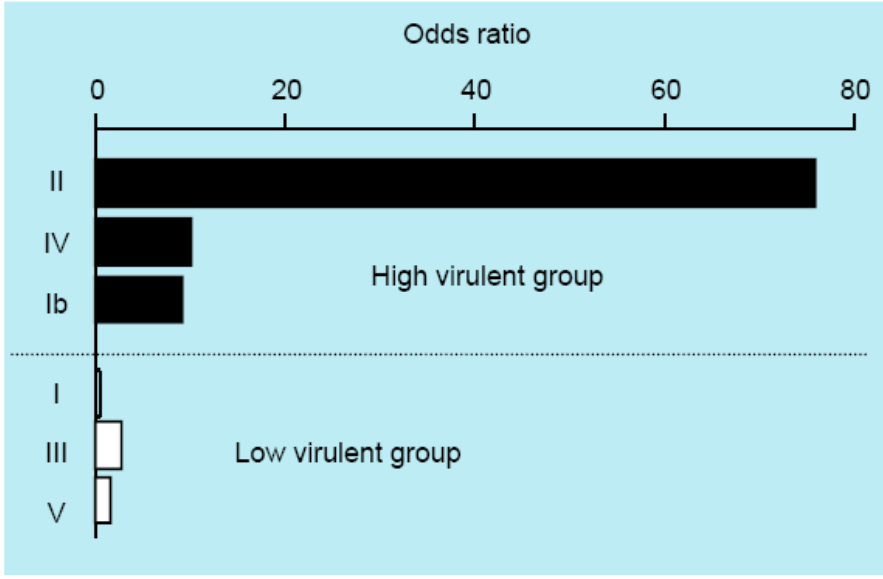

Figure 24. Odds ratios of the *P. gingivalis fimA* genotypes and marginal periodontitis based on the *fimA* genotypes distribution in marginal periodontitis patients.

A total of 650 saliva specimens were isolated from 464 children (3 to 18 years of age) were analyzed, and PCR detection showed that only 15 (3.23%) subjects were *P. gingivalis*-positive [72] (Figure 25). The detection ratesfor*P. gingivalis* and the ages of the subjects were similar to those in a previous study [47, 48] which supports the suggestion that *P. gingivalis* tended to be more frequently detected in older subjects. It should be noted that *P. gingivalis* is regarded as a transient species in children and adolescents and the detection rates for*P. gingivalis*at different times was shown to be 25-67% in *P. gingivalis*-positive subjects [48]. Therefore, multiple specimens at different times are required to identify*P. gingivalis*-positive subjects. Furthermore, additional assaysof these specimens to discriminate between the *fimA* genotypes demonstrated that none of these showed a positive reaction to the type II *fimA*-specific primers, while 4, 1, and 2 subjects were shown to be positive for the type I, Ib, and III genotypes, respectively. In addition, the type IV genotype was detected in three subjects in the older age group. It should be noted that one-third of the *fimA* genotypes were determined to be untypeable in *P. gingivalis*-positive subjects. It remains to be elucidated whether the

untypeable specimens contained only a single or multiple unknown *fimA* genotypes. However, it is possible that this/these genotypes possibly belong to low virulence groups, such as I, III and V. Taken together, these results suggest that the distribution of type II and IV *fimA* genotypes is extremely low in children although only a limited number of children do harbor *P. gingivalis* with low virulence for periodontitis. Furthermore, some adolescents were found to possess the type IV *fimA* genotype, which are possibly related to the onset of marginal periodontitis.

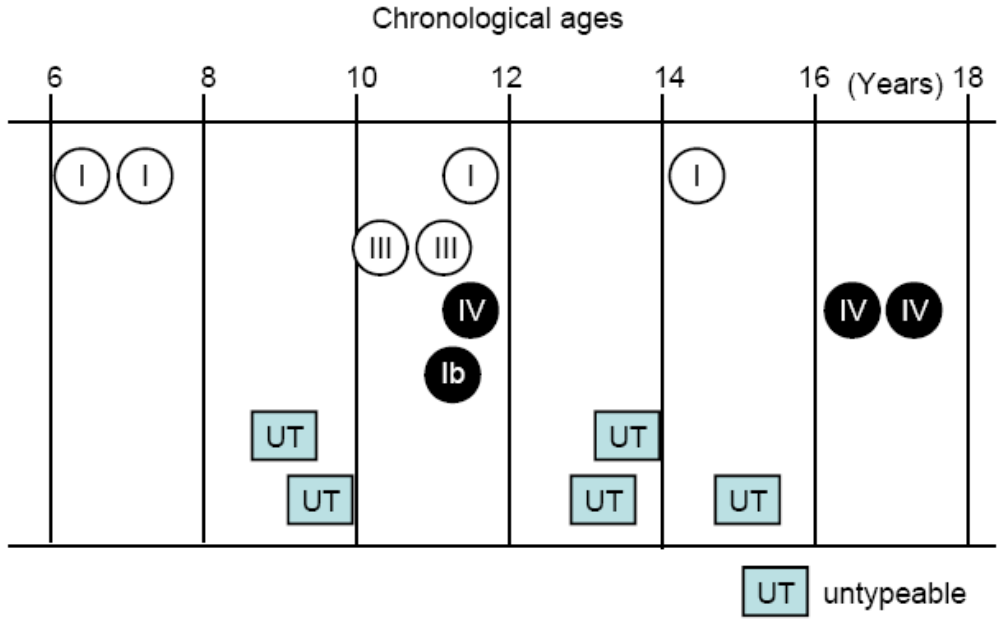

Figure 25. Detection of *P. gingivalis* and determination of *fimA* genotypes in children and adolescents. Open and closed circles indicate the low and high virulence types, respectively.

7) Down's Syndrome Subjects

Down's syndrome (DS) is known to be a genetic disease resulting from trisomy of the 21st chromosome, occurring in 1 out of 600-1000 births [86, 87]. It is widely known that subjects with DS often develop extensive gingivitis at early stages and exhibit extensive rapid and generalized periodontal breakdown in early adulthood, which is estimated to result from impaired immune responses, fragile periodontal tissue and early senescence [18, 88]. Subjects with DS are commonly encountered in daily dental practice; however, there is little information on periodontal bacterial species using molecular biological techniques in these subjects. Thus, we decided to analyze the bacterial species in DS subjects.

The distribution of periodontal species in 60 children with Down's syndrome (2 to 13 years old, 5 in each age bracket) was compared with those of 60 age-matched systemically healthy controls [89]. There were no obvious signs of marginal periodontitis in the DS group and no significant clinical difference from the control group. PCR detection of the 10 periodontitis-related species in subgingival plaque specimens showed that most of the pathogens were detected with greater frequency in the DS children than in the controls

(Figure 26). *T. denticola*, *T. forsythia*, *P. nigrescens*, and *C. rectus* were significantly prevalent throughout all age brackets of the DS children. The occurrence of *P. gingivalis* was also significant in the DS subjects over 5 years old. These results demonstrated that important pathogens for several types of adult periodontitis, such as *P. gingivalis*, *T. forsythia* and *T. denticola* [52], are frequently found in DS groups. Although these species are considered to be transient in systemically healthy children, early colonization could occur in the DS group. In addition, the severity of gingivitis was associated with increased varieties of the resident pathogens as well as the distribution of *P. gingivalis.*Analyses of subgingival plaque specimens from DS patients also showed that early-onset periodontitis in DS is mainly due to the increased susceptibilityof the hostto the causative microbial agents including *P. gingivalis* with type II *fim*Agenes. Furthermore, it was demonstrated that gingival fibroblasts from subjects with DS were impaired significantly by *P. gingivalis* type II FimA as compared to those from systemically healthy subjects [90]. This impairment is likely due to invasion of *P. gingivalis* readily leading to impaired cellular motility, which is estimated to prevent wound healing and the regeneration of periodontal tissues.

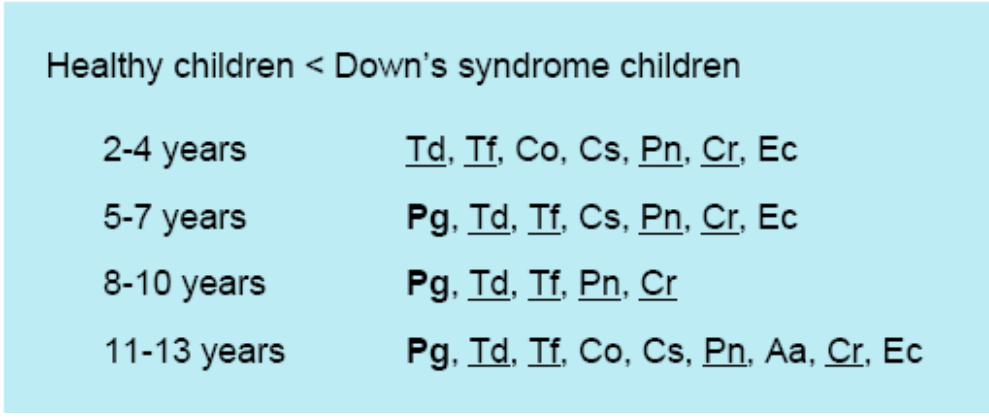

Figure 26. Periodontal bacterial species are detected in significantly higher numbers in children with Down's syndrome as compared to those with systemically healthy children. Bold letters indicate the common species in the children more than 5 years old. Underlines indicate the common species identified in all age groups.

8) Children with Developmental Disabilities

Maintenanceof good oral hygiene is generally considered to be difficult in subjects with developmental disabilities.This leads to speculation that the distribution of periodontal speciesis different in these subjects as compared to systemically healthy individuals. However, there are few reports describing these species in children with developmental disabilities. Therefore, we determined the distribution of the periodontal bacterial species in subjects attending daycare centers due to the developmental disabilities [91]. A total of 187 children (136 boys, 51 girls) aged 1-6 years attending daycare centers, were analyzed. They were diagnosed withmental retardation (MR), cerebral palsy (CP), autism (AU), or pervasive developmental disorders. Dental plaque specimens were collected from the buccal side of the maxillary left second primary molar. PCR analyses demonstrated that *C. sputigena* was the

most frequently detected species (28.3%), followed by *A. actinomycetemcomitans* (20.9%) and *C. rectus* (18.2%). *E. corrodens*, *C. ochracea*, and *P. nigrescens* were detected in approximately 10% of the specimens, whereas *T. denticola*, *T. forsythia*, and *P. intermedia* were rarely found, and *P. gingivalis* was not detected in any of the subjects. The mean value for the total number of the 10 tested bacterial species in all subjects was 1.16 species, which was positively correlated with age. In addition, the total numbers of detected species were positively correlated with the age of the subjects (Figure 27), with a maximum of 8 species identified in one subject.

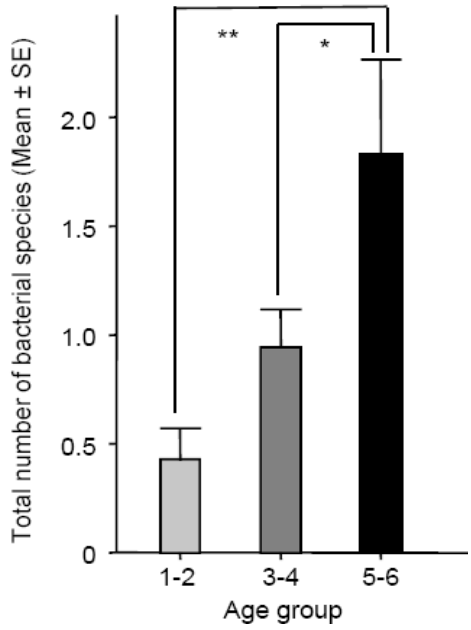

Figure 27. Total number of 10 periodontal bacterial species in each age group of children with developmental disabilities. There were statistical significant differences among them (*P<0.05, **P<0.01).

There were 10 subjects with positive reactions for *T. denticola* and/or *T. forsythia*, in whom the total number of bacterial species was significantly higher as compared to the other subjects. Furthermore, subjects possessing *C. rectus* showed significantly greater values for periodontal pocket depth, gingival index, and total number of species, indicating that *C. rectus* is one of the possible indicators for risk of periodontitis. On the other hand, the clinical parameters evaluating periodontitis were shown to be worse in the group of subjects complicated with concomitant MR, CP, and AU. The total number of the species detected in this group of subjects was significantly higher than those of the other groups complicated with a single or two concomitant diseases of MR, CP, and AU (Figure 28). It is reasonable to understand that individuals with multiple developmental disabilities have great difficulties with maintaining oral health and undergoing dental treatments as compared to subjects with a single disability. In addition, the present results showed that one-fourth of the subjects with disabilities were shown to possess at least one of the periodontitis-associated species (*T. denticola*, *T. forsythia*, and *C. rectus*)and should also be regarded as possible subjects at risk for the onset of periodontitis. These subjects are currently receiving special periodical

professional oral health care. Periodical PCR detection of the species could be beneficial for evaluating their current oral status and to estimate their future risk for the onset of periodontitis.

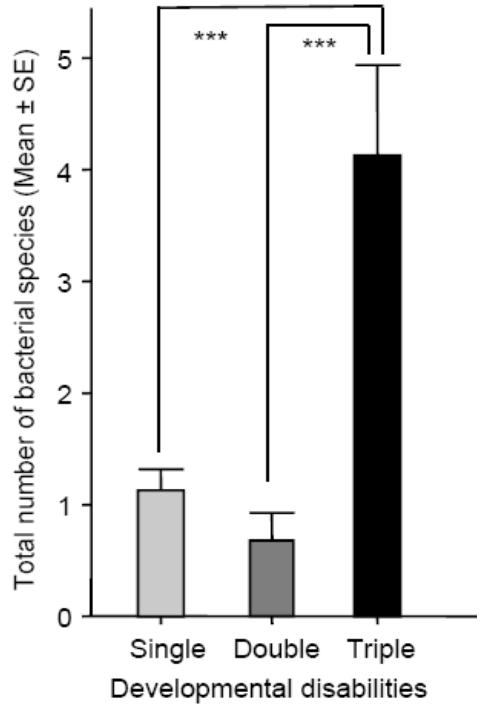

Figure 28. Total number of 10 periodontal bacterial species in groups classified on the basis of the number of the complicated disabilities. There were statistical significant differences among them (***P<0.001).

Conclusion

The clinical features of periodontal diseases in children and adolescents are different from those of adults. Gingivitis is very common in children, and professional tooth brushing instruction and/or removal of dental calculusallows for the recovery of healthy gingiva for a short period of time. In contrast, periodontitis is rarely identified in systemically healthy children, and, if found, may require consultation with pediatricians to evaluate complications from systemic diseases. The prognoses for periodontitis in these patients is poor due to a background of abnormal immune responses. On the other hand, the number of subjects with periodontitis gradually increases with age during adolescence. Early diagnosis and appropriate interventions are necessary in order to prevent the transition to the typical marginal periodontitis observed in adults.

Considering the etiology of periodontitis, the analysis of periodontal bacterial species in clinical specimens appears to be important. The recent development of molecular biological techniques enables the rapid detection of periodontal bacteria in clinical specimens. For the past decade, we have evaluated the distribution of periodontal bacterial species in children, the interannual changes of the species in the same subjects, the combinations of species

detected simultaneously, and mother-to-child transmission in Japanese children using molecular biological techniques. In addition, the distribution of these bacterial species in children with Down's syndrome and other developmental disabilities was also analyzed. Using these and other approaches,we will initiatelongitudinal monitoringof periodontal species, clinical application of these methods, including risk assessmentfor future periodontitis, and evaluation of the effects of daily periodontal treatment in the near future.

References

[1] Jenkins, W. M. and Papapanou, P. N. (2001). Epidemiology of periodontal disease in children and adolescents. *Periodontol. 2000 26*, 16-32.

[2] Armitage, G. C. (1999). Development of a classification system for periodontal diseases and conditions. *Ann. Periodontol. 4*, 1-6.

[3] Silness, J. and Löe, H. (1964). Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odoltol. Scand.22*, 121-135.

[4] Löe, H. and J. Silness, J. (1963). Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontol. Scand.21*, 533-551.

[5] Hart, T. C., Pallos, D., Bozzo, L., Almeida, O. P., Marazita, M. L,. O'Connell, J. R. and Cortelli, J. R. (2000). Evidence of genetic heterogeneity for hereditary gingival fibromatosis. *J. Dent. Res. 79*, 1758-1764.

[6] Miyamoto, E., Nakano, K., Nomura, R., Fujita, K., Okawa, R. and Ooshima, T. (2008). Evaluation of transitional changes of sub-gingival bacterial species in twins with gingival fibromatosis. *Ped. Dent. J. 18*, 57-63.

[7] Seymour, R. A., Ellis, J. S. and Thomason, J. M. (2000). Risk factors for drug-induced gingival overgrowth. *J. Clin. Periodontol. 27*, 217-223.

[8] Vernillo, A. T. and Schwartz, N. B. (1987). The effects of phenytoin (5,5-diphenylhydantoin) on human gingival fibroblasts in culture. *J. Periodontol. Res. 22*, 307-312.

[9] Stinnett, E., Rodu, B. and Grizzle, W. E. (1987). New developments in understanding phenytoin-induced gingival hyperplasia. *J. Am. Dent. Assoc. 114*, 814-816.

[10] Hassell, T. M. and Hefti, A. F. (1991). Drug-induced gingival overgrowth: Old problem, New problem. *Crit. Rev. Oral Biol. Med. 2*, 103-137.

[11] Thomason, J. M., Seymour, R. A. and Rice, N. (1993). The prevalence and severity of cyclosporin and nifedipine-induced gingival overgrowth. *J. Clin. Periodontol. 20*, 37-40.

[12] Majola, M. P., McFadyen, M. L., Connolly, C., Nair, Y. P., Govender, M., Laher, M. H. (2000). Factors influencing phenytoin-induced gingival enlargement. *J. Clin. Periodontol. 27*, 506-512.

[13] Akiyama, S., Amano, A., Kato, T., Takada, Y., Kimura, K. R. and Morisaki, I. (2006). Relationship of periodontal bacteria and *Porphyromonas gingivalis fimA* variations with phenytoin-induced gingival overgrowth. *Oral Dis. 12*, 51-56.

[14] Nakano, K., Nishiyama, N., Tamura, K, Sasaki, H. and Ooshima, T. (2004). Clinical and microbiological evaluations of gingival fibromatosis in children: Report of two cases. *Ped. Dent. J. 14*, 141-146.

[15] Ooshima, T., Takiguchi, M., Tamura, K. and Nishiyama, N. (2002). Clinical and microbiological changes in a child with rapid alveolar bone loss and refill. *ASDC J. Dent. Child. 69*, 143-147.

[16] Tamura, K., Nakano, K., Miyake, S., Takada, A. and Ooshima, T. (2005). Clinical and microbiological evaluations of acute periodontitis in areas of teeth applied with orthodontic bands. *Ped. Dent. J. 15*, 212-218.

[17] Ooshima, T., Nishiyama, N. and Tamura, K. (2003). Accidentally induced periodontitis in primary dentition: longitudinal examinations of periodontal bacteria and clinical conditions. *Int. J. Paediatr. Dent. 13*, 193-197.

[18] Meyle, J. and Gonzáles, J. R. (2001). Influences of systemic diseases on periodontitis in children and adolescents. *Periodontol. 2000 26*, 92-112.

[19] Hu, C. C., King, D. L., Thomas, H. F. and Simmer, J. P. (1996). A clinical and research protocol for characterizing patients with hypophosphatasia. *Pediatr. Dent.18*, 17-23.

[20] Fallon, M. D., Teitelbaum, S. L., Weinstein, R. S., Goldfischer, S., Brown, D. M. and Whyte, M. P. (1984). Hypophosphatasia: clinicopathologic comparison of the infantile, childhood, and adult forms. *Medicine63*, 12-24.

[21] El-Labban, N. G., Lee, K. W. and Rule, D. (1991). Permanent teeth in hypophosphatasia: light and electron microscopic study. *J. Oral Pathol. Med.20*, 352-360.

[22] Miyamoto, E., Nakano, K., Tamura, K., Nomura, R., Sasaki, Y. and Ooshima, T. (2007). Clinical and microbiological evaluations of children with hypophosphatasia affected by periodontitis. *Ped. Dent. J. 17*, 84-92.

[23] Everett, F. G. and Kramer, G. M. (1972). The disto-lingual groove in the maxillary lateral incisor; A periodontal hazard. *J. Periodontol.43*, 352-361.

[24] Withers, J. A., Brunsvold, M. A., Killoy, W. J. and Rahe, A. J. (1981). The relationship of palato-gingival grooves to localized periodontal disease. *J. Periodontol.52*, 41-44.

[25] Kogon, S. L. (1986). The prevalence, location and conformation of palato-radicular grooves in maxillary incisors. *J. Periodontol.57*, 231-234.

[26] Peikoff, M. D., Perry, J. B. and Chapnick, L. A. (1985). Endodontic failure attributable to complex radicular lingual groove. *J. Endod.11*, 573-577.

[27] Robison, S. F. and Cooley, R. L. (1988). Palatogingival groove lesions: recognition and treatment. *Gen. Dent.36*, 340-342.

[28] Mayne, J. R. and Martin, I. G. (1990). The palatal radicular groove. Two case reports. *Aust. Dent. J. 35*,277-281.

[29] Goon, W. W., Carpenter, W. M., Brace, N. M. and Ahlfeld, R. J. (1991). Complex facial radicular groove in a maxillary lateral incisor. *J. Endod.17*, 244-248.

[30] Cecilia, M. S., Lara, V. S. and de Moraes, I. G. (2001). The palato-gingival groove. A case of failure in root canal treatment. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.85*, 94-98.

[31] Meister, F. Jr., Keating, K., Gerstein, H. and Mayer, J. C. (1983). Successful treatment of a radicular lingual groove: Case report. *J. Endod.* 9, 561-564.

[32] Andreana, S. (1998). A combined approach for treatment of developmental groove associated periodontal defect. A case report. *J. Periodontol.69*, 601-607.

[33] Nakano, K., Miyamoto, E., Nemoto, H., Nomura, R., Shintani, S. and Ooshima, T. (2007). Clinical and microbiological evaluations of mandibular lateral incisor with radicular-gingival groove. *Ped. Dent. J.17*, 93-99.

[34] Moore, W. E. (1987). Microbiology of periodontal disease. *J. Periodontal Res.22*, 335-341.

[35] van Winkelhoff, A. J. and de Graaff, J. (1991). Microbiology in the management of destructive periodontal disease. *J. Clin. Periodontol.18*, 406-410.

[36] Kroes, I., Lepp, P. W. and Relman, D. A. (1999). Bacterial diversity within the human subgingival crevice. *Proc. Natl. Acad. Sci. USA 96*, 14547-14552.

[37] Kolenbrander, P. E. (2000). Oral microbial communities: biofilms, interactions, and genetic systems. *Annu. Rev. Microbiol. 54*, 413-437.

[38] Paster, B. J., Boches, S. K., Galvin, J. L., Ericson, R. E., Lau, C. N., Levanos, V. A., Sahasrabudhe, A. and Dewhirst, F. E. (2001). Bacterial diversity in human subgingival plaque. *J. Bacteriol. 183*, 3770-3783.

[39] Ashimoto, A., Chen, C., Bakker, I. and Slots, J. (1996). Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. *Oral Microbiol. Immunol.11*, 266-273.

[40] Conrads, G., Mutters, R., Fischer, J., Brauner, A., Lütticken, R. and Lampert, F. (1996). PCR reaction and dot-blot hybridization to monitor the distribution of oral pathogens within plaque samples of periodontally healthy individuals. *J. Periodontol.67*, 994-1003.

[41] Tran, S. D. and Rudney, J. D. (1996). Multiplex PCR using conserved and species-specific 16S rRNA gene primers for simultaneous detection of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*. *J. Clin. Microbiol.34*, 2674-2678.

[42] Watanabe, K. and Frommel, T. O. (1996). *Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans* and *Treponema denticola* detection in oral plaque samples using the polymerase chain reaction. *J. Clin. Periodontol.23*, 212-219.

[43] Doungudomdacha, S., Rawlinson, A. and Douglas, C. W. (2000). Enumeration of *Porphyromonas gingivalis*, *Prevotella intermedia* and *Actinobacillus actinomycetemcomitans* in subgingival plaque samples by a quantitative-competitive PCR method.*J. Med. Microbiol.49*, 861-874.

[44] Rovery, C., Greub, G., Lepidi, H., Casalta, J. P., Habib, G., Collart, F. and Raoult, D. (2005). PCR detection of bacteria on cardiac valves of patients with treated bacterial endocarditis. *J. Clin. Microbiol. 43*, 163-167.

[45] Marques da Silva, R., Caugant, D. A., Eribe, E. R., Aas, J. A., Lingaas, P. S., Geiran, O., Tronstad, L. and Olsen, I. (2006). Bacterial diversity in aortic aneurysms determined by 16S ribosomal RNA gene analysis. *J. Vasc. Surg. 44*, 1055-1060.

[46] Kuboniwa, M., Amano, A., Kimura, K. R., Sekine, S., Kato, S., Yamamoto, Y., Okahashi, N., Iida, T. and Shizukuishi, S. (2004). Quantitative detection of periodontal pathogens using real-time polymerase chain reaction with TaqMan probes. *Oral Microbiol. Immunol. 19*, 168-176.

[47] Kimura, S., Ooshima, T., Takiguchi, M., Sasaki, Y., Amano, A., Morisaki, I. and Hamada, S. (2002). Periodontopathic bacterial infection in childhood. *J. Periodontol.73*, 20-26.

[48] Ooshima, T., Nishiyama, N., Hou, B., Tamura, K., Amano, A., Kusumoto, A. and Kimura, S. (2003). Occurrence of periodontal bacteria in healthy children: a 2-year longitudinal study. *Community Dent. Oral Epidemiol.31*, 417-425.

[49] Amano, A., Kishima, T., Kimura, S., Takiguchi, M., Ooshima, T., Hamada, S. and Morisaki, I. (2000). Periodontopathic bacteria in children with Down syndrome. *J. Periodontol. 71*, 249-255.

[50] Alaluusua, S. and Asikainen, S (1988). Detection and distribution of *Actinobacillus actinomycetemcomitans* in the primary dentition. *J. Periodontol.9*, 504-507.

[51] Riviere, G. R., Smith, K. S., Tzagaroulaki, E., Kay, S. L., Zhu, X., DeRouen, T. A. and Adams, D. F. (1996). Periodontal status and detection frequency of bacteria at sites of periodontal health and gingivitis. *J. Periodontol.67*, 109-115.

[52] Darveau, R. P., Tanner, A. and Page, R. C. (1997). The microbial challenge in periodontitis. *Periodontol. 200014*, 12-32.

[53] Wara-aswapati, N., Howell, T. H., Needleman, H. L. and Karimbux, N. (1999). Periodontitis in the child and adolescent. *ASDC J. Dent. Child66*, 167-174.

[54] Lamell, C. W., Griffen, A. L., McClellan, D. L. and Leys, E. J. (2000). Acquisition and colonization stability of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in children. *J. Clin. Microbiol.38*, 1196-1199.

[55] Darby, I. and Curtis, M. (2001). Microbiology of periodontal disease in children and young adults. *Periodontol. 200026*, 33-53.

[56] Nakano, K., Miyamoto, E., Tamura, K., Nemoto, H., Fujita, K., Nomura, R. and Ooshima, T. (2008). Distribution of 10 periodontal bacterial species in children and adolescents over a 7-year period. *Oral Dis. 14*, 658-664.

[57] Socransky, S. S., Haffajee, A. D., Cugini, M. A., Smith, C. and Kent, R. L. Jr. (1998). Microbial complexes in subgingival plaque. *J. Clin. Periodontol.25*, 134-144.

[58] Rôças, I. N., Siqueira, J. F. Jr., Santos, K. R. and Coelho, A. M. (2001). "Red complex" (*Bacteroides forsythus*, *Porphyromonas gingivalis*, and *Treponema denticola*) in endodontic infections: A molecular approach. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.91*, 468-471.

[59] Suda, R., Kurihara, C., Kurihara, M., Sato, T., Lai, C. H. and Hasegawa, K. (2003). Determination of eight selected periodontal pathogens in the subgingival plaque of maxillary first molars in Japanese school children aged 8-11 years. *J. Periodontal Res.38*, 28-35.

[60] Suda, R., Kobayashi, M., Nanba, R., Iwamaru, M., Hayashi, Y., Lai, C. H. and Hasegawa, K. (2004). Possible periodontal pathogens associated with clinical symptoms of periodontal disease in Japanese high school students. *J. Periodontol.75*, 1084-1089.

[61] Rickard, A. H., Palmer, R. J. Jr., Blehert, D. S., Campagna, S. R., Semmelhack, M. F., Egland, P. G., Bassler, B. L. and Kolenbrander, P. E. (2006). Autoinducer 2: a concentration-dependent signal for mutualistic bacterial biofilm growth. *Mol. Microbiol.60*, 1446-1456.

[62] Kreth, J., Merritt, J., Shi, W. and Qi, F. (2005). Competition and coexistence between *Streptococcus mutans* and *Streptococcus sangunis* in the dental biofilm. *J. Bacteriol.187*, 7193-7203.

[63] Kuramitsu, H. K., He, X., Lux, R., Anderson, M. H. and Shi, W. (2007). Interspecies interactions within oral microbial communities. *Microbiol. Mol. Biol. Rev.71*, 653-670.

[64] Tamura, K., Nakano, K., Hayashibara, T., Nomura, R., Fujita, K., Shintani, S. and Ooshima, T. (2006). Distribution of 10 periodontal bacteria in saliva samples from Japanese children and their mothers. *Arch. Oral Biol.51*, 371-377.

[65] Miyamoto, E., Nakano, K., Fujita, K., Nomura, R., Okawa, R., Matsumoto, M. and Ooshima, T. (2009). Bacterial profiles of oral streptococcal and periodontal bacterial species in saliva specimens from Japanese subjects. *Arch. Oral Biol. 54*, 374-379.

[66] Lapirattanakul, J., Nakano, K., Nomura, R., Hamada, S., Nakagawa, I. and Ooshima, T. (2008). Demonstration of mother-to-child transmission of *Streptococcus mutans* using multilocus sequence typing. *Caries Res. 42*, 466-474.

[67] Kozai, K., Nakayama, R., Tedjosasongko, U., Kuwahara, S., Suzuki, J., Okada, M. and Nagasaka, N. (1999). Intrafamilial distribution of mutans streptococci in Japanese families and possibility of father-to-child transmission. *Microbiol. Immunol. 43*, 99-106.

[68] Alaluusua, S., Asikainen, S. and Lai, C. H. (1991). Intrafamilial transmission of *Actinobacillus actinomycetemcomitans*. *J. Periodontol.62*, 207-210.

[69] Petit, M. D., van Steenbergen, T. J., Scholte, L. M., van der Velden, U. and de Graaff, J. (1993). Epidemiology and transmission of *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* among children and their family members. A report of 4 surveys. *J. Clin. Periodontol.20*, 641-650.

[70] Kamma, J. J., Diamanti-Kipioti, A., Nakou, M. and Mitsis, F. J. (2000). Profile of subgingival microbiota in children with mixed dentition. *Oral Microbiol. Immunol.15*, 103-111.

[71] Könönen, E., Wolf, J., Mättö, J., Frandsen, E. V., Poulsen, K., Jousimies-Somer, H. and Asikainen, S. (2000). The *Prevotella intermedia* group organisms in young children and their mothers as related to maternal periodontal status. *J. Periodontal Res.35*, 329-334.

[72] Tamura, K., Nakano, K., Nomura, R., Miyake, S., Nakagawa, I., Amano, A. and Ooshima, T. (2005). Distribution of *Porphyromonas gingivalis fimA* genotypes in Japanese children and adolescents. *J. Periodontol. 76*, 674-679.

[73] Umeda, M., Miwa, Z., Takeuchi, Y., Ishizuka, M., Huang, Y., Noguchi, K., Tanaka, M., Takagi, Y. and Ishikawa, I. (2004). The distribution of periodontopathic bacteria among Japanese children and their parents. *J. Periodontal Res. 39*, 398-404.

[74] Watson, M. R., Bretz, W. A. and Loesche, W. J. (1994). Presence of *Treponema denticola* and *Porphyromonas gingivalis* in children correlated with periodontal disease of their parents. *J. Dent Res. 73*, 1636-1640.

[75] Amano, A. (2003). Molecular interaction of *Porphyromonas gingivalis* with host cells: Implication for the microbial pathogenesis of periodontal disease. *J. Periodontol. 74*, 90-96.

[76] Amano, A., Kuboniwa, M., Nakagawa, I., Akiyama, S., Morisaki, I. and Hamada, S. (2000). Prevalence of specific genotypes of *Porphyromonas gingivalis fimA* and periodontal health status. *J. Dent. Res.79*, 1664-1668.

[77] Nakagawa, I., Amano, A., Kimura, R. K., Nakamura, T., Kawabata, S. and Hamada, S. (2000). Distribution and molecular characterization of *Porphyromonas gingivalis* carrying a new type of *fimA* gene.*J. Clin. Microbiol. 38*, 1909-1914.

[78] Nakagawa, I., Amano, A., Ohara-Nemoto, Y., Endoh, N., Morisaki, I., Kimura, S., Kawabata, S. and Hamada, S. (2002). Identification of a new variant of *fimA* gene of *Porphyromonas gingivalis* and its distribution in adults and disabled populations with periodontitis. *J. Periodontal Res.37*, 425-432.

[79] Amano, A., Nakagawa, I., Okahashi, N. and Hamada, N. (2004). Variations of *Porphyromonas gingivalis* fimbriae in relation to microbial pathogenesis. *J. Periodontal Res.39*, 136-142.

[80] Beikler, T., Peters, U., Prajaneh, S., Prior, K., Ehmke, B. and Flemmig, T. F. (2003). Prevalence of *Porphyromonas gingivalis fimA* genotypes in Caucasians. *Eur. J. Oral Sci. 111*, 390-394.

[81] Missailidis, C. G., Umeda, J. E., Ota-Tsuzuki, C., Anzai, D. and Mayer, M. P. (2004). Distribution of *fimA* genotypes of *Porphyromonas gingivalis* in subjects with various periodontal conditions. *Oral Microbiol. Immunol. 19*, 224-229.

[82] van der Ploeg, J. R., Giertsen, E., Lüdin, B., Mörgeli, C., Zinkernagel, A. S. and Gmür, R. (2004). Quantitative detection of *Porphyromonas gingivalis fimA* genotypes in dental plaque. *FEMS Microbiol. Lett. 232*, 31-37.

[83] Nakagawa, I., Amano, A., Kuboniwa, M., Nakamura, T., Kawabata, S. and Hamada, S. (2002). Functional differences among FimA variants of *Porphyromonas gingivalis* and their effects on adhesion to and invasion of human epithelial cells. *Infect. Immun.70*, 277-285.

[84] Nakano, K., Kuboniwa, M., Nakagawa, I., Yamamura, T, Nomura, R., Okahashi, N., Ooshima, T. and Amano, A. (2004). Comparison of inflammatory changes caused by *Porphyromonas gingivalis* with distinct *fimA* genotypes in a mouse abscess model. *Oral Microbiol. Immunol. 19*, 205-209.

[85] Kato, T., Kawai, S., Nakano, K., Inaba, H., Kuboniwa, M., Nakagawa, I., Tsuda, K., Omori, H., Ooshima, T., Yoshimori, T. and Amano, A. (2007). Virulence of *Porphyromonas gingivalis* is altered by substitution of fimbria gene with different genotype. *Cell. Microbiol.* 9, 753-765.

[86] Desai, S. S. (1997). Down syndrome: A review of the literature. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 84*, 279-285.

[87] Roizen, N. J. and Patterson, D. (2003). Down's syndrome. *Lancet 361*, 1281-1289.

[88] Reuland-Bosma, W. and van Dijk, J. (1986). Periodontal disease in Down's syndrome: A review. *J. Clin. Periodontol. 13*, 64-73.

[89] Amano, A., Kishima, T., Akiyama, S., Nakagawa, I., Hamada, S. and Morisaki, I. (2001). Relationship of periodontopathic bacteria with early-onset periodontitis in Down's syndrome. *J. Periodontol. 72*, 368-373.

[90] Murakami, J., Kato, T., Kawai, S., Akiyama, S., Amano, A. and Morisaki, I. (2008). Cellular motility of Down syndrome gingival fibroblasts is susceptible to impairment by *Porphyromonas gingivalis* invasion. *J. Periodontol. 79*, 721-727.

[91] Naka, S., Yamana, A., Nakano, K., Okawa, R., Fujita, K., Kojima, A., Nemoto, H., Nomura, R., Matsumoto, M. and Ooshima, T. (2009). Distribution of periodontopathic bacterial species in Japanese children with developmental disabilities. *BMC Oral Health 9*, 24.

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Chapter III

Biomechanics of Rehabilitating the Perioprosthetic Patient

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Abstract

In advanced perioprosthetic cases where the periodontium's integrity is severely compromised and the dental barrier's function is extremely disrupted, the biomechanical response to the extrinsic mechanical stimuli of the system including the prosthetic restoration supported by the biological tissues is quite altered. The differentiated altered experience of the functional loading due to the lowered periodontium's threshold along with the apical shift of the system fulcrum due to the periodontium's structure reduction require a modified design of the restoration's metal framework as a critical factor in the system's survival in order to secure the expected longevity of both the restorative and biological structures, capturing the failure initiation of either progressive tissular or technical collapse. So, the purpose of the present study was to: a. analyze the way by which the periodontium reacts to the developing forces and how its integrity is related to the experience of the stress field on the perioprosthetic patient; b. determine the parameters defining the tooth prognosis in the perioprosthetic patient and how the restoration type is involved; c. report the clinical significance of tooth splinting by cantilever cross arch fixed partial denture applied on the perioprosthetic patient and the way it is related to the response of the reduced periodontium and finally d. investigate the

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clinical significance of the specific design of the metal framework in cantilever cross-arch fixed partial dentures via a theoretical finite element model.

A. Prognosis and Perioprosthetics

Treatment planning for the perioprosthetic patient is an extremely challenging task due to the specificity of the pathogenesis, concerning an oral disability, characterized by the structural deterioration of physiological tissues and the disruption of their normal function. This case is induced by the attack on periodontal tissues of generalized moderate or advanced periodontitis, which leads to marked periodontal lesion and subsequent loss of varied amount of tissue that scales up to more than 50% of the existing normal peridontium. [1] Insufficient dental support –in regard to tooth function impairment-, constitutes the dominant problem for both the perioprosthetic patient and the dentist –as this makes rehabilitation of the dentition increasingly difficult (Figure 1). [2, 3]

The current therapeutic approach of the perioprosthetic patient aims at the successful establishment of the health of the periodontal tissues and the subsequent restoration of the normal function of, not only the teeth but also of the mouth disturbed function after accomplishing the periodontal therapy (Figure 2). Both periodontology and prosthodontics are therefore involved in the planning of the treatment and, depending on the severity and pattern of the lesions, the participation of professionals from other dental specializations (orthodontics, endodontics, dental surgery) could be deemed imperative. This is a highly complicated and time consuming process which involves frequent reassessment of the therapy plan at each one of its stages, based on the continuous assessment of the prognosis for the tooth. Therefore, the prognosis for the tooth survival defines the therapeutic treatment, hence it constitutes the most imperative and at the same time the most controversial part of perioprosthetics. It is carried out within the framework of a procedure for forecasting the tooth survival over time, assessing concrete parameters within the framework of choice, not within the framework of a therapeutic prosthetic shape which transforms and from which is being transformed simultaneously. The identification of a risk level for the disease progression defines the frequency and the range of supportive therapy, while simultaneously averts situations of hypo- or –over-treatment.

In the literature, different prognostic models are proposed which try to foresee the tooth survival, based on parameters which are defined either during the initial therapy phase or during the supportive therapy phase. Those parameters regard the characteristics which are located at the genetic level, at the tooth level, at the patient level, even at the socio-economic level. [6, 7, 8, 9, 10, 11, 12] These studies try to establish a correlation between the prognosis and the tooth survival (tooth loss), while simultaneously try to correlate those parameters with the tooth loss, which presents the final phase of periodontal disease.[13] However, while these parameters have been recorded and assessed, they do not seem to have been correlated accurately with the tooth survival. McGuire et al. (1996, 1999), in a series of articles, certify the obvious relation between the clinical parameters used for the initial prognosis and the tooth survival. Nevertheless, they point out that this relation is not accurate and cannot be described. At the same time, they assert that, while the parameters used for the prognosis foresee to a sufficient degree the tooth survival with the initially good prognosis, yet the same is not valid for teeth categorized as teeth with the prognosis less than a good one. [14, 15, 16,

17]Moreover, McLeod et al. (1998) declare that the prognosis of teeth that are initially characterized as doubtful, is not that precise as the prognosis of teeth which are initially characterized as teeth with a bad prognosis. [18] The above-mentioned study concurs with that of by Fardal et al. (2004). These results are to confirm those largeretrospective studies which report a very small rate of tooth loss in advanced periodontal patients after being under follow-up programs for several years. Specifically, in a study of Hirschfeld and Wasserman (1978), 600 treated periodontal patients, which were systematically re-examined for more than 15 years, lost 4 teeth only in total, while in relative studies a very small rate of tooth loss in periodontal patients during a long period of reexamining is referred. [19, 20, 21, 22, 23] Wilson et al. (1987), in a comparative study of tooth loss during the period of more than 5 years in treated periodontal patients, which had systematically followed the program of supportive therapy and in patients who failed to follow that program, point out that the highest tooth loss occurred in patients that didn't follow the program. [24] Certainly, it is mentioned in literature that the compliance of patients to the program of re-examination is not correlated with a tooth loss in patients which suffer from a moderate or an initial periodontitis. [25, 26] To an extent at which there are always doubts with regard to the relativity of the above-mentioned results, these studies prove that the prognosis is a dynamic procedure which is constantly being altered, precisely because the clinical parameters used for the tooth survival are being affected by the periodontal treatment or by the additional supportive treatment.[27] However, although there are some parameters which can be eliminated very easily (bleeding during an examination, residual periodontal pockets) or difficultly (smoking), there are some other parameters which are irreversible and concern the tooth loss and the periodontal support loss. [28] In perioprosthetic patients, given the degree of the previous osseous destruction, the progressively increasing mobility, the tooth loss and the possibility of the disease to progress in a persistent or a recurrent type, the prognosis is of crucial significance, since the treatment is accompanied by extendedcross-arch, fixed restorations. [29, 30] However, despite the fact that the ability of the severely reduced periodontal tissues to withstand those restorations had been questioned in the past, breaking the Ante's law, there are studies which corroborate that, despite the loss of periodontal support at a degree above 50%, the prognosis of those teeth is good. [31, 32] Moreover, the results of Nyman and Lindhe (1979) and Nyman and Ericsson's studies (1982) prove that limitations of the implementation of fixed restorations in abutment teeth with an extremely reduced periodontium are attributed to technical and bio-mechanical problems in relation to the construction of restorations, rather than to the biological ability of the periodontium to support them successively. Of course, this is applied on the condition that strict protocol of preservation of the sub-therapeutic program is followed. [33] The position of abutment teeth, the presence of endodontic treatment, the restoration type and the type of the opposite dental barrier had been implicated as factors aggravating the prognosis in perioprosthetic patient. [34] However, results of clinical studies prove the good, for the time period over than 5 years, prognosis of teeth with a healthy but extremely reduced periodontium as abutments of extensive fixed prosthetic restorations (cross-arch splinting with or without the use of cantilevers), decoherencing the restoration type and the tooth position with the prognosis. [35, 36, 37]

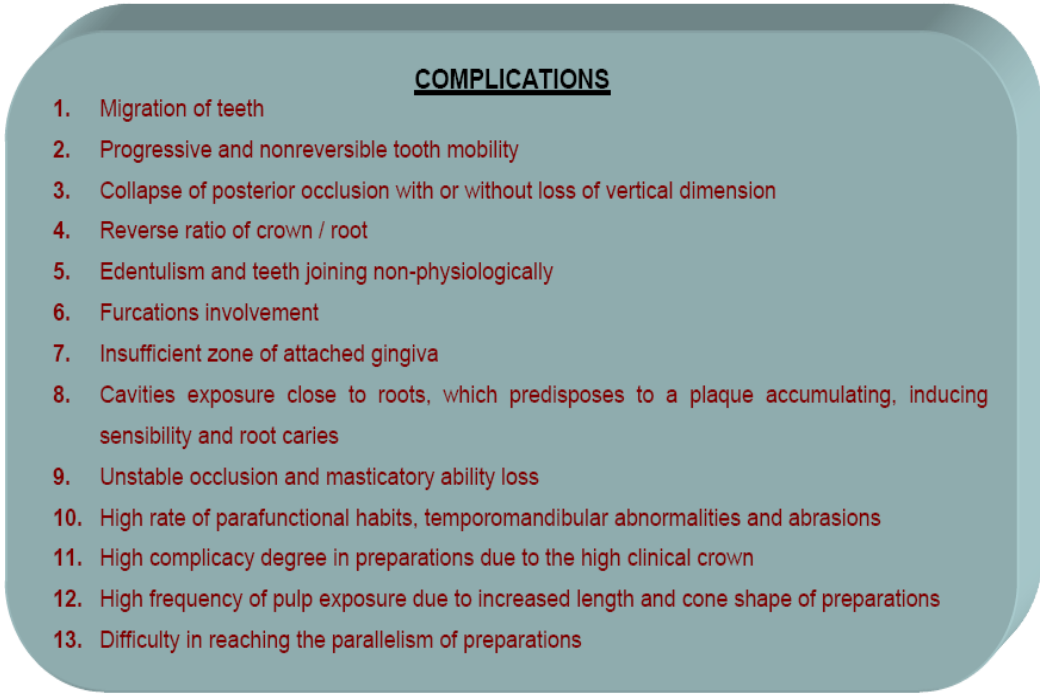


Figure 1. Problems of perioprosthetic patient[3].

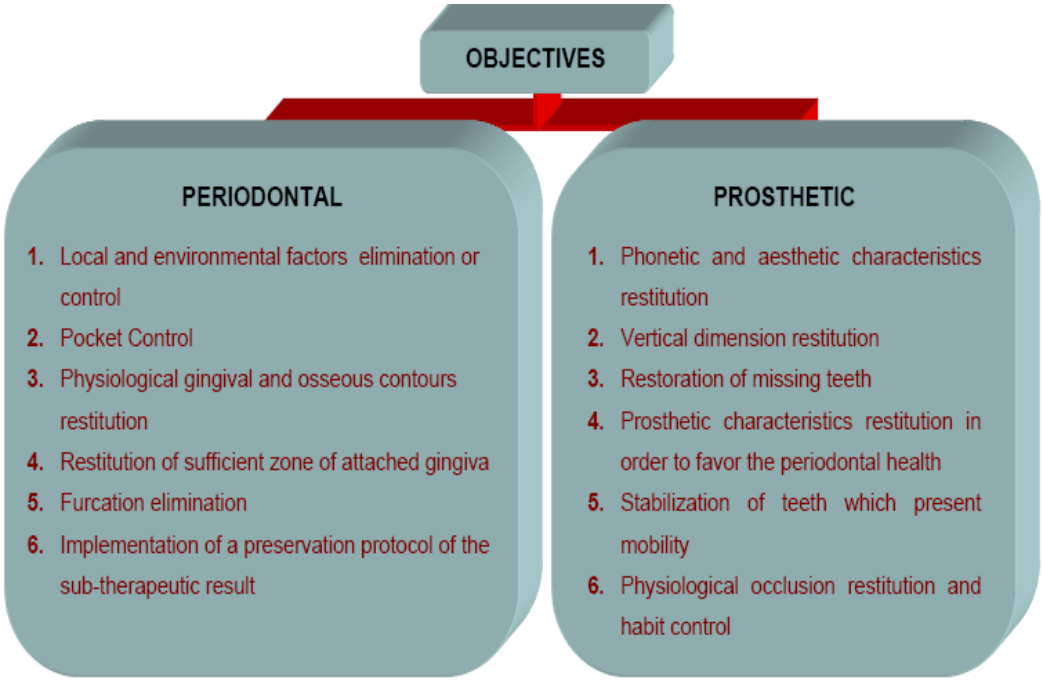


Figure 2. Objectives of perioprosthetic therapy [4].

B. The Reaction of Periodontal Tissues to the Developing Forces

The structural and the metabolic stability of the periodontal ligament depend on its mechanical stimulation by occlusal forces, creating a system in a dynamic balance. However, this balance can be frequently diverted to a pathological – adaptable state of periodontal tissues as a result of developing stresses, under the level of their endurance. [38] In the literature,the above-mentioned diversion is referred to as an occlusal trauma and is divided into primary and secondary. The primary trauma regards the tissue reaction which is enabled when the supporting tissues have the physiological height, whereas the secondary trauma regards the case when the periodontal tissues are reduced, thus even the physiological forces enable the same tissue reaction of the trauma. [39] In particular, in primary occlusal trauma the muscle contraction is basically isometric, and the loading forces excel 250 lb/sq in, whereas in secondary trauma the muscle contraction is basically isotonic, and the forces fluctuate between 2-15 lb/sq in. [40] However, precisely because the differentiation is based only in quantitative difference of the height of periodontal tissues, in modern bibliography the differentiation of the two types of occlusal trauma is considered needless and consequently not used. [41]

The role of occlusal trauma in pathogenesis of the periodontal disease is anissue under research from the beginning of the 20th century, when Karolyi (1901) determined originally the interdependence between the high occlusal loads and the alveolar "pyorrhea". [42] Despite the fact that the research activity during the 1960's had already concluded that force transmission in the periodontium brings about physiological and pathological changes to the alveolar bone and the periodontal ligament, it has not yet been proven that these changes may cause the friction loss. [43, 44, 45, 46, 47] Since then, quite many studies were carried out on autopsy material [48], on experimental animals and humans, at the histological and clinical level [49-56], exploring the impact of the occlusal trauma on the initiation, progression and treatment of periodontal disease. However, these studies could not clarify the role of the trauma in osseous destruction, arriving at conclusions which had been unreliable and often controversial first of all because there are no experimental animals which imitate the human stomatognathic system [57], and secondly, because the well-planned clinical trials could not be accomplished with a control group due to the absence of both criteria and reliable indices for the existence and the degree of periodontal trauma caused by occlusion. [41, 58] Also, different methods for causing both the experimental periodontitis and the occlusal trauma have been accused of different results. [39]

The research groups of Polson et al. and Lindhe et al. have carried out experimental studies on squirrel monkeys and beagle dogs respectively, exploring the impact of the occlusal trauma on periodontal tissues when the periodontal disease does not exist. Both groups arrive at the conclusion that high occlusal loading during the absence of periodontal disease cause mobility and osseous support loss. More specific, the bone loss correlates with the widening of periodontal ligament, whereas in some cases the horizontal osseolysis is occurred. [49, 50] Polson (1976a) points out that neither separate nor jiggling forces can cause friction loss, irrespective of the presence of apparent histological changes in the alveolar bone and the periodontal ligament, whereas the jiggling forces can possibly cause the

reduction of the alveolar bone height, but can mostly cause the loss of the total bone volume. [59]

Nevertheless, the two groups arrive to some extent at different conclusions as to the impact of the occlusal trauma on periodontal tissues in the presence of periodontal disease. The possibility of synergy between the occlusal trauma and the periodontitis progression is an opinion that had been formulated by Glickman, [60] whereas many following studies tried to substantiate the above-mentioned hypothesis, although without any of them having reached to complete the synergy criteria of Glickman. [45, 61, 62] Lindhe et al. correlate the acceleration of the attachment loss with high occlusal loads, and the growth of the force meter with the irreversibility of developing changes. [52, 63, 64]The group of Polson et al. considered that the correlation between the attachment loss degree and the occlusal trauma is insignificant in the presence of periodontal disease, whereas the elimination of trauma cannot reduce the mobility or the loss of osseous support, if the inflammation control does not precede. However, teeth which had not undergone the occlusal trauma in the presence of an existing periodontitis, present the greatest bone loss in relation to the control group, as it concurs with the studies of Lindhe. [65, 66, 67, 68]

Clinical trials with human tissue are very restricted. The majority of them try to correlate the clinical and the radiographic variables which have been considered (but had not been verified) as symptoms of occlusal trauma (mobility, widening of the periodontium, and type of occlusal contacts) with the severity of periodontal disease, without though being able to formulate clearly whether it is progression indices or an existence of periodontal disease. Svanberg et al. (1995) point out that excessive loading does not necessarily cause tooth mobility, because the duration and the frequency (not the force meter) are significant to cause mobility. Suitable criteria for the clinical diagnosis of trauma caused by occlusion are the simultaneous existence of increasing mobility and the widening of the periodontium, whereas the clearest difference of the increasing from stabilized mobility is a histological existence of the active osseous lysis and inflammation in case of increasing mobility. [69]

Philstrom et al. (1986), carrying out a study of the first molars of 300 humans, have not observed any correlation between the pocket depth, the clinical attachment level, and the percentage of the osseous support in relation to the type of occlusal contacts. Their study concurs with results of the study of Shefter and McFall who have not found any correlation between the two parameters either. [55, 70] However, a study on human material, using the diagnostic casts to diagnose abrasions, points out the increased mobility, the pocket depth and the bone loss in relation to interventions in the not-working side. [71] Additionally, it should be mentioned that in the study of Philstrom et al. (1986) it had been observed that those teeth considered having affection by the occlusal trauma, had the highestpocket depth, attachment loss and osseous support loss. Trying to precisely explore the contribution of the trauma to the loss of osseous support, a regression analysis had been carried out, during which it had been confirmed that in two tooth groups with the same attachment loss and pocket depth, from which the one had an additional mobility and widenedperiodontium, the loss of the osseous support had been by 10% more compared to teeth without trauma symptoms. [55] The results of the above-mentioned study concur with those that result from the study of Jin and Cao (1992), with a difference that during the regression analysis of Philstrom et al. it had been observed that the percentage of osseous loss between the control group and the under examination group is always stable regarding the level of attachment loss, whereas Jin and

Cal had observed that the rate of osseous loss for teeth with trauma signs is higher for every higher level of the attachment loss. [56]

Houston et al. (1987) have conducted a study, in which, trying to correlate the periodontal disease with bruxism, confirmed that there is no any correlation between the two parameters, [72] as it concurs with the relevant study of Budtz-Jorgensen (1980). [73] Hakkarainen et al. (1986), studying in patients the gingival fluid flow after the elimination either of periodontal disease or the premature contacts, observed that the reduction of gingival fluid is achieved only after the elimination of inflammation. [74]

The review of bibliographic data till 1996 of the trauma role in periodontal disease has concluded that scientific data bases is possibly insufficient in order to determine a clear correlation between the two parameters, whereas the ethical restrictions also make difficult the planning of progressive studies with the control group, which could clarify the situation. [75]

In a modern bibliographic actuality Numm (2001) and Harrel (2001), considering the impact of the occlusal interventions on periodontal disease, carried out studies on human material, where they tried to correlate the occlusal status both with clinical parameters, which are demonstrative of the periodontal disease, and with the progression of periodontitis. In these studies it is reported that the role of the trauma in periodontal disease is underestimated, whereas a strong correlation between the occlusal trauma and the periodontal destruction is confirmed, verifying that the trauma is an independent risk factor in periodontal disease. [76, 77]

C. Splinting and Perioprosthetics

The problem of the occlusal trauma in perioprosthetic patient, as it is determined by the above-mentioned clinical and experimental studies, seems to have at least a correlation to the bone loss, as it becomes apparent by a state of increased mobility. This mobility, according to Nyman and Lang (1994), is explained by the fact that in advanced stages of periodontal disease, the level of supportive tissues is located in such a place, that, despite the preceding periodontal treatment and the occlusal balancing, the remaining periodontal tissues are not able to endure the masticatory forces. [78] In these cases the only way to inhibit the following destruction of the periodontal apparatus is to immobilize teeth by means of a cross-arch restoration. [79]

The primary function of splinting consists in, through the immobilization of teeth, rearrangement of the lateral forces to as more as possible vertical forces – parallel to the longitudinal axis of the tooth – which are more endurable in comparison with periodontal tissues, due to the greater number of fibrous bundles of the root cementum. This rearrangement is achieved by the transformation of splinted teeth to a unified system with a common rotation center, which is located in the splint center, far from the root cementum of each tooth. [80] As mentioned above, the splinting alters the type and the distribution of developing stresses in tissues, but this does not mean that even the force meter changes. Hence, whereas some lateral traumatic forces are neutralized by some others in the immobilization system, reducing the developing stresses in some teeth, other teeth included in the splint can simultaneously accept the same or even higher stresses in comparison with what

it had been prior to the immobilization. [81] Moreover, the teeth immobilization which is achieved by splinting consists in a reduction or even an elimination of mobility only for the period when a splint is applied. More concretely, in a Renggli and Schverzer's study (1974) it is reported that those teeth splinted with a view to reducing their mobility, reacquired their mobility at almost the same level as it had been prior to the splinting during a period of approximately6 monthsafter splint removal. [82] In a similar study with a control group consisting of patients without splinted teeth had been explored the reduction of mobility of splinted teeth with telescopic crowns, which had been preserved in their position for 4 weeks. After the fourth till the tenth week the splinting had been removed during the regular periods of time in order to measure the tooth mobility. No statistically substantial differences were noted between the two groups. This fact is rather expected, because after splint removal, the same conditions reappear (biological state and load conditions), which had created the occlusal trauma. [83]

Though, it should be mentioned that beyond the impact to occlusal trauma, currently splinting is chosen even in cases of stabilized mobility, which, whereas is not indicative of the occlusal trauma and the following periodontal destruction, however affects the masticatory ability and the stabilization of occlusal contacts, the comfort and the aesthetics of the patient, the destabilization of which is raised from the collapse of occlusion. [23, 40]Considering the prosthetic options for patients treated for advanced periodontal diseasethe cross-arch FPD ofshortened dental arch concept is considered to be a conservative prosthodontic treatment,[84-86] while splinting with cantilever cross-arch-designed FPD may be preferred since extension to the molar area seems to provide improved esthetics, masticatory function, occlusal stability, and properly directed occlusal forces. [87,88]

In the past, the splinting in perioprosthetic patient had been a controversial issue due to the use of teeth with an extremely reduced but otherwise healthy periodontium as abutments in extensive restorations, what contradicted to previous dominating views, which were based on the Ante's Law. [89] However, Nyman and Ericsson (1982) have proven that the ability of teeth with an extremely reduced periodontium (10% of the total surface of periodontal tissues for a dental barrier) to support the fixed cross-arch restorations had been the same good in relation to the teeth with a periodontium not so reduced after 8-11 years. [32]

In a series of studies carried out in order to record and to evaluate complications related to the fixed cross-arch restorations in perioprosthetic patient, it was reported that they were mostly of technical nature, not of biological / periodontal nature, and were related to the presence of catnilevers. [90]

In 1986, Randow et al. presented a study in which they recorded the technical and biological complications in three patient groups, which had been treated by fixed cross-arch restorations without cantilevers, with one cantilever or with two cantilevers, respectively. In this study they confirmed a direct correlation between the addition of cantilevers and the rate of technical complications, which was increased over time basically for the group with the two cantilevers. The rate of complications has almost doubled when an additional cantilever was added, reaching 44% for restorations extending in double cantilevers bilaterally. Basic technical complications were the retention loss and the restoration fracture. These complications were basically related to the end-abutment, whereas the restoration fracture was mostly located in the area of proximal connector of the end-abutment. Furthermore, a significant correlation between the complications and the vitality of the end-abutment is accentuated, as it concurs with the studies of Nyman (1979), Karlsson (1984), Sorensen

(1985), and Lindquist (1998), and is explained by the fact that the end-abutment undergoes a systematic concentration of excessive stresses when it is not alive. [33, 34, 91, 92] On the contrary, the end-abutments in fixed cross-arch restorations with cantilevers show the greatest possibility of necrosis, as it is confirmed by other studies.[93-96] Randow's group (1986) also accentuates the same correlation, which is considered significant basically for restorations with two cantilevers and is justified by an extensive cut ofdental substance during the preparation of the end-abutment in order to ensure the parallelism of walls for the optimisation of retention. [34] However, other research groups present far less rates of both technical and biological complications during the restoration with the fixed splinting with cantilevers. Nyman and Lyndhe (1979), in a study similar to that of Randow's (1986), recorded 8% of technical complications after 5-8 years of observation, irrespective of the presence of cantilevers. This study concurs with the study of Randow as regards the complication type, however this rate corresponds to the complication rate that Randow et al. attribute to restorations without cantilevers after 7 years of observation. [33] The results of the above-mentioned study correspond to those of Lundgren and Laurell's, which presented a very small rate of complications for fixed restorations extending bilaterally in double or triple cantilevers. [97, 98] Likewise, in a three-year study of fixed cross-arch restorations with cantilevers in Koreans, Yi et al. (2001) found respective results. [37] In essence, the last studies formulate the view, according to which the splinting type, with regard to the presence of cantilevers, does not affect the restoration prognosis. However, it should be mentioned that the small rate of the last studies is attributed to special modifications conducted in relation to the connectors' dimensions of the metal framework, the type of preparations and of occlusion, as well as the strict therapy protocol and the repeated examination that had been followed. More precisely, the length and the width of the connectors nearly doubled in comparison with the normal range (length: 5-6, width 4-5), the teeth preparations (mostly the end-abutment) became parallel as far as possible, whereas the occlusal contacts were retrieved along the full length of the dental arc without interventions in working or non-working side. [99] The proper shaping of occlusion is a significant factor for the prognosis of restoration with cantilevers. However, when it is not possible (patients with the skeletal Class II), it is better to avoid the use of cantilevers. In particular, experimental studies have proven that the absence of contact in the anterior area of a fixed cross-arch restoration with cantilevers induces stress concentration in the area of cantilevers and the increase of the bendingmoments at the point of junction with the end-abutment. Likewise, experimental studies carried out by Laurell and Falk's research groups point out that the restitution of equivalent contacts with the rest dental barrier causes a favourable distribution of stresses along the length of cantilevers. Specifically, the forces that develop in the second cantilever are approximately one sixths compared to those that develop in the first cantilever, what is explained by the root apex bending deflection of the second cantilever in relation to the first. [100] However, premature contacts of about 80 nm induce the reverse distribution of stresses, presenting a risk factor for the restoration. [101] Finally, a factor which is considered significant for the prognosis of restoration with cantilevers is the type of a competitor barrier. However, even in this case, opinions seem to be conflicting, because Falk (1989) records an increase of dynamic fields in the area of cantilevers when the competitor barrier is presented by the complete denture, despite the fact that Randow et al. (1986), in a clinical study, have not found any statically significant difference between the technical complications and the type of an opposing dental arch. [34, 102]

As shown above, the implementation of the fixed cross-arch restoration with cantilevers may present a therapeutic reality with a very good prognosis, on the condition that indispensable prerequisites occur as regards the therapeutic and the restorative part.

D. Biomechanics of Cross-Arch Fixed Partial Dentures in Perioprosthetic Patient/Technical and Biological Aspect

Clinical studies reporting on success rates of extensive restorations for severely reduced periodontal tissues present higher failure rates than for no-cantilever fixed prostheses. [103, 104]The complications arising are mechanical rather than biological, primarily related to fracture of the connectors. [35]

Connectors seem to be regions of high stress concentration and, therefore, frequently involve complications. [105] Previous clinical studies have demonstrated increased rates of failure when connectors with conventional dimensions were used for extended cross-arch FPDs with minimum periodontal tissue support, especially proximal to the retaining abutment. [34, 106]Thus, dimensioning of connectors is a critical factor in the survival of cantilever cross-arch FDPs in the perioprosthetic patient, as its importance is enhanced by theconsiderable biomechanical demands. [107]In details, reduction of the osseous support moves the fulcrum of the tooth apically. This results in high stress concentrations within the connectors due to a large rotary vertical vector with long leverage for each tooth. [107] This stress increase renders connectors prone to failure, regardless of the material used, since they are the weakest points of the prosthesis due to their relative small cross-section to the overall construction. [108]The stress concentration within the connectors is further enhanced by the addition of a cantilever segment. [108]Proper dimensioning of the connectors was established by mathematical calculations derived from a formula used by Erhardson. [109] The results obtained indicate that the technical safety of the cantilevered FDPs is ensured by increasing the vertical dimension (VD) of the connectors adjacent to the cantilever segment to 5 to 6 mm and their horizontal dimension (HD) to 4 to 5 mm. [99] Clinical studies, incorporating this formula in the fabrication of cantilever cross-arch FDPs in the perioprosthetic patient, report lower technical failure rates compared to studies in which the dimensions of the connectors were lesser. [33-35, 96, 99, 104, 110]

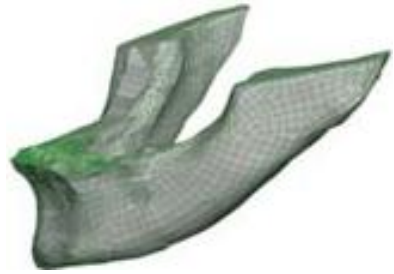
The positive outcomes obtained from such therapeutic regimens have been reported primarily in clinical studies conducted in Scandinavian countries. [36, 37] In this population, the anatomic average of crown diameters could incorporate the increase of the connectors to these dimensions, [111] while the posterior teeth were restored using complete cast crowns or pontics. However, the increased use of metal ceramic restorations in the entire restored dental arch, in combination with the smaller mesiodistal and buccolingual diameters recorded for the teeth of other human population groups, renders such dimensions not always attainable. [37, 112] Consequently, even if the elongated clinical crown resulting from advanced periodontal disease permits the increased height of connectors, the HD is limited by the tooth size and the type of prosthetic restoration. Therefore, the question arises as to how the biomechanical behavior of the connectors is affected when an increase in VD is not combined with a simultaneous increase in HD.

Testing the biomechanical performance of the connectors related to their dimensions in clinical studies is not easy because it is difficult to standardize the dimensions of the connectors. [113, 114] Therefore, the analysis of the biomechanics of the connectors has been studied primarily using theoretical methods. [81, 109, 110, 113, 115-126]This emphasizes the importance of proper *insilico* studies. The most sophisticated theoretical method for simulating clinical reality is Finite Element Analysis (FEA), [20] an *in silico* numerical tool predicting biomechanical response. [21]This has the advantage over clinical studies of reducing the number of uncontrolled variables influencing the final outcome. [20]Finite element analysis (FEA) may solve such complicated design problems, [127-129] using the principles of engineering and material science. [130, 131] However, few FEA studies are available, [124, 126] and the authors identified no studies pertaining to the optimal dimension of the connectors proximal to the retaining abutment of cross-arch FDPs, extended as cantilever segments, with minimal osseous support. Consequently, in research activity of the Department of Fixed Prosthesis and Implant Prosthodontics, Aristotle University of Thessaloniki, *in silico* studies have been conducted using Finite Element Analysis Software in order to investigate and optimize the biomechanics of the metal-framework regarding to technical and biological integrity of cross-arch FPDs in the perioprosthetic patient.

The methodology followed was based on the evaluation of digital parametric anatomic models, derived from a 3-D basic one. All the structures were either obtained from a Computed Tomography (CT) image processing system (MIMICS: Materialise Interactive Medical Image Control System; Materialise N.V., Leuven, Belgium) or developed in 3-D Computer-Aided Design (CAD) (Solidworks 2006; Solidworks Corp, Concord, Mass) and Reverse Engineering (RE) (Geomagic Studio; Geomagic Inc, North Carolina) environments.

The original model simulated a human adult mandible, dentate bilaterally to the second premolars, with a normal height of alveolar bone (Figure 3, A and B).

A.



B.

Figure 3. Original Model. A. Mandible, B. Teeth.

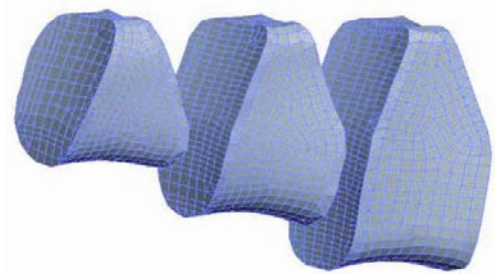


Figure 4. Connectors proximal to the end-abutment, adjacent to cantilever segment. 3, 4, 5 mm-connectors in vertical dimension.

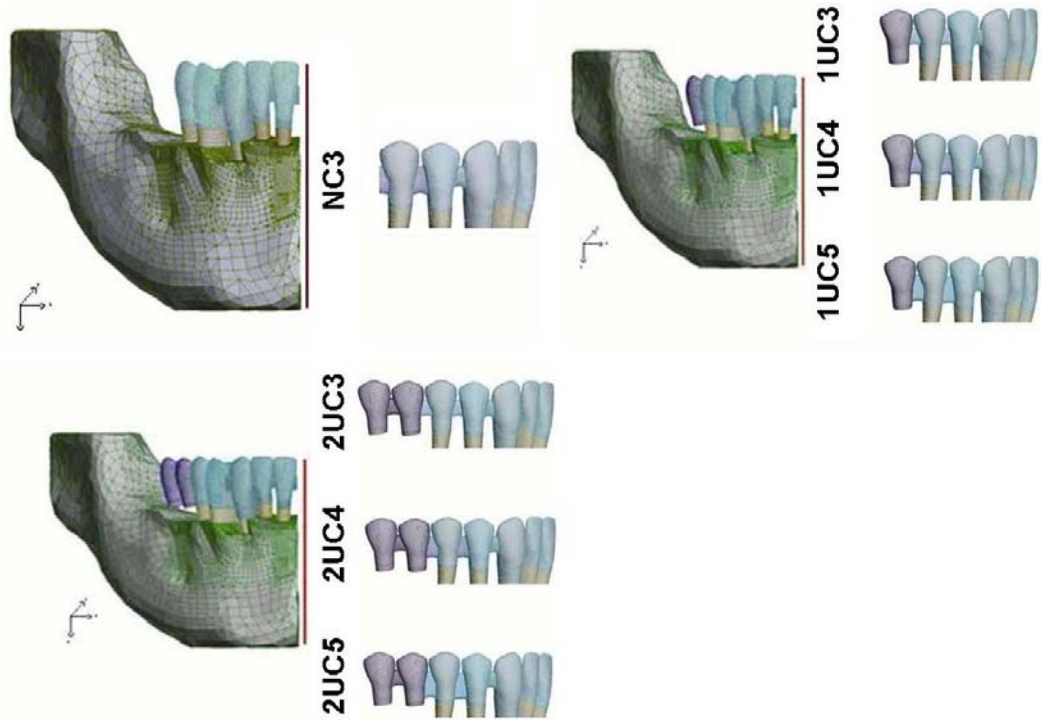


Figure 5. Models. NC3: No-cantilever FPD, 1UC3: 1-unit cantilever FPD, 3mm-connector, 2UC3: 2-unit cantilever FPD, 3mm-connector, 1UC4: 1-unit-cantilever FPD, 4mm-connector, 2UC4: 2-unit cantilever FPD, 4mm-connector, 1UC5: 1-unit cantilever FPD, 5mm-connector, 2UC5: 2-unit cantilever FPD, 5mm-connector.

The original model was modified to form parametric models restored with a cross-arch FPD: a. not extended-without cantilevers and b. extended bilaterally as 1- or 2- unit cantilevers. The VD of the connectors proximal to retaining abutment of the extended FPDs was investigated for the values 3 (conventional), 4 and 5 mm, while their HD remained stable at 2,5mm (Figure 4). [132]

Each type of restoration was investigated at 50% of reduced bone support. [7]The resulting models and their symbols are shown in Figure 5.

Table 1. Maximum von Mises stress values (MPa) on teeth for each model

Models		Maximum Von Mises Stress Values (MPa)				
Osseous Support	Teeth	Central incisor	Lateral incisor	Canine	First premolar	Second premolar
	Models					
50%	NC	3.10	3.20	3.40	14.50	13.10
	1UC3	5.10	5.03	5.75	9.78	35.22
	2UC3	5.74	7.42	7.38	10.48	63.32
	1UC4	5.04	5.05	5.68	10.40	32.7
	2UC4	5.80	7.67	7.45	10.63	56.42
	1UC5	4.99	5.01	5.62	11.30	31.8
	2UC5	4.18	9.70	16.11	23.22	135.62

Table 2. Maximum von Mises stress values (MPa) on teeth for each model

Models		Maximum Von Mises Stress Values (MPa)				
Osseous Support	Periodontal ligaments	Central incisor	Lateral incisor	Canine	First premolar	Second premolar
	Models					
50%	NC	0.912	0.468	0.576	1.740	4.900
	1UC3	0.955	0.642	0.489	1.730	8.220
	2UC3	0.957	0.781	0.498	1.680	9.580
	1UC4	0.947	0.634	0.488	1.760	8.220
	2UC4	0.948	0.766	0.486	1.720	9.650
	1UC5	0.941	0.624	0.475	1.770	8.220
	2UC5	0.823	0.656	0.451	1.970	10.160

All parametric models were subsequently imported into the FEA program, where a linear static analysis was performed (Algor; Algor Inc, Pittsburg, Pa). The adjustment analysis' parameters (boundary conditions, loading case and materials properties) for the FEA analysis

was defined. [108, 133]Stress distribution patterns of the connectors, the periodontal ligaments and the teeth were obtained by calculating von Mises equivalent stresses and described in Figures 6-8. The relative stress values for the connectors are described elsewhere, [133] while the stress values for the periodontal ligaments and teeth are shown in Tables 1 and 2.

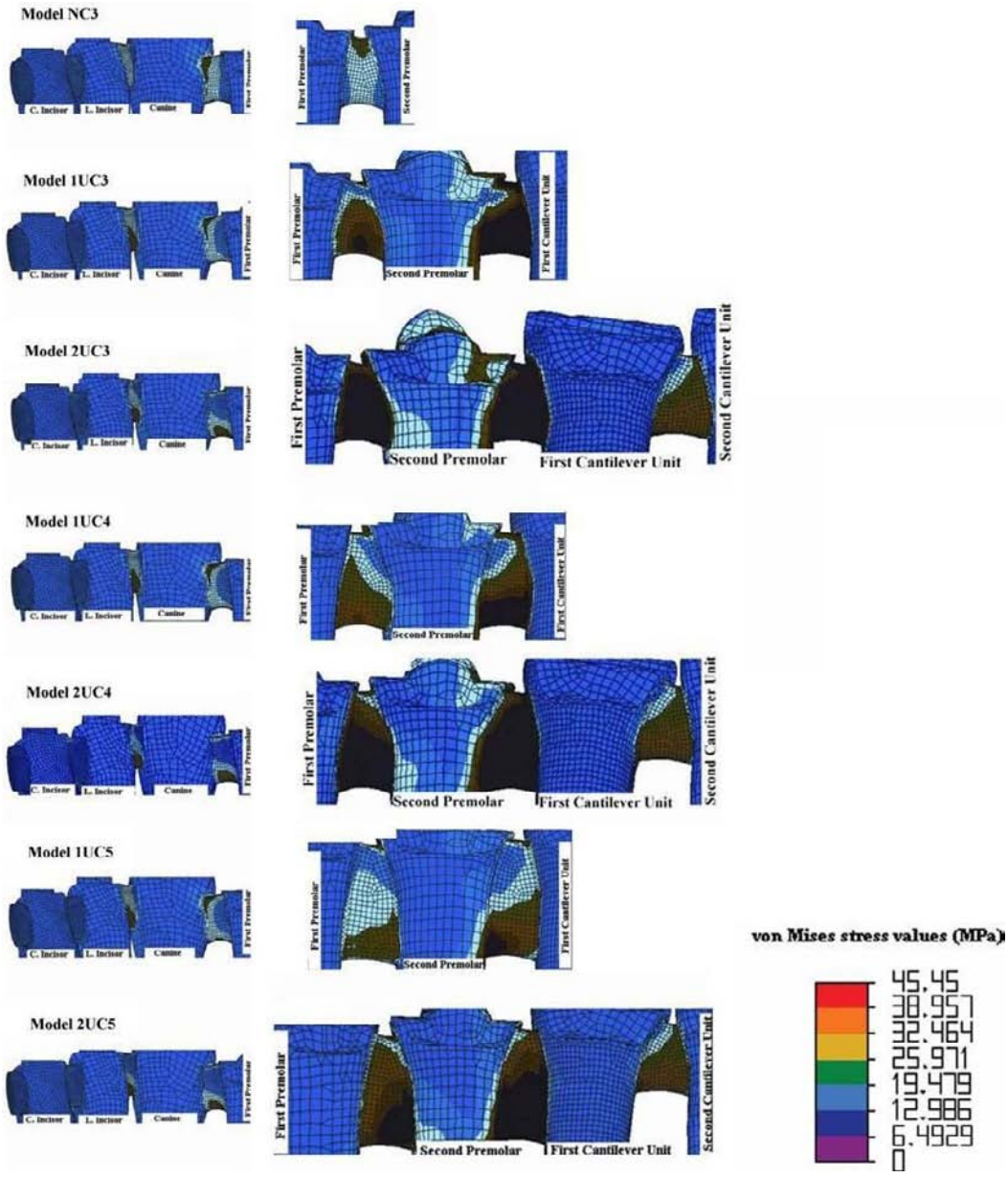


Figure 6. Stress distribution of the connectors: NC3: No-cantilever FPD, 1UC3: 1-unit cantilever FPD, 3mm-connector, 2UC3: 2-unit cantilever FPD, 3mm-connector, 1UC4: 1-unit-cantilever FPD, 4mm-connector, 2UC4: 2-unit cantilever FPD, 4mm-connector, 1UC5: 1-unit cantilever FPD, 5mm-connector, 2UC5: 2-unit cantilever FPD, 5mm-connector, colored measurement bar (MPa).

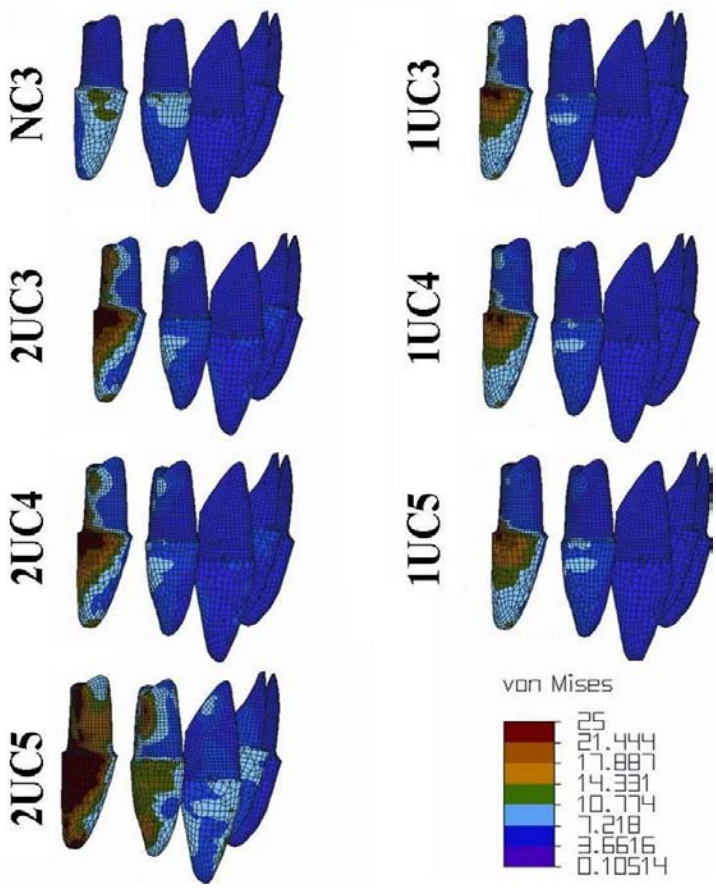


Figure 7. Stress distribution of the teeth of the models: NC3: No-cantilever FPD, 1UC3: 1-unit cantilever FPD, 3mm-connector, 2UC3: 2-unit cantilever FPD, 3mm-connector, 1UC4: 1-unit-cantilever FPD, 4mm-connector, 2UC4: 2-unit cantilever FPD, 4mm-connector, 1UC5: 1-unit cantilever FPD, 5mm-connector, 2UC5: 2-unit cantilever FPD, 5mm-connector, colored measurement bar (MPa).

The present study suggests that the higher stress concentration is located within the connectors and the areas of splinted crowns around the connectors,while both 1- and 2- unit cantilever restorations presented the highest stress values proximal to the retaining abutment, independently of connectors VD. More specific, the higher stress values were reported for the connectors distal to the retaining abutment, while the stress values calculated for the connector distal to the retaining abutment of the 2-unit cantilever restoration were almost doubled compared to the connector of the 1-unit cantilever restoration. These outcomes are in accordance with the results derived from the FEA studies of Awadalla (1992), Yang (1996) and Wang (1998) as well as the photoelastic study of Wylie and Caputo (1991), who investigated theoretical models of FPDs with conventional connectors. [81, 118-121] However, to authors' knowledge, there are no relevant studies investigating the biomechanical effect of varying the connectors VD of cross-arch FPDs in the perioprosthetic patient, in order to allow for a comparative assessment of this study's results.

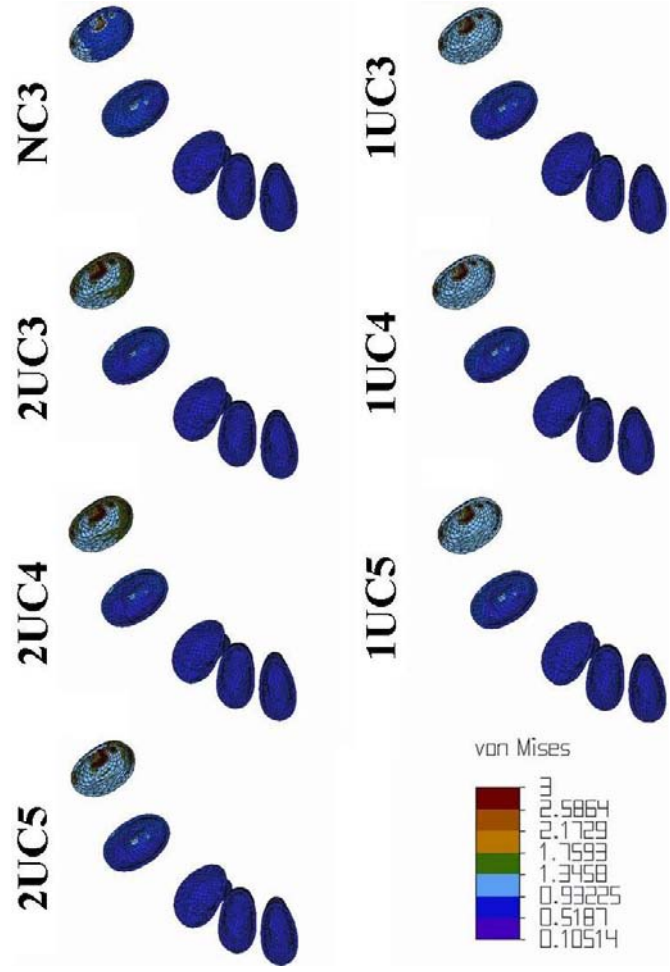


Figure 8. Stress distribution of the periodontal ligaments: NC3: No-cantilever FPD, 1UC3: 1-unit cantilever FPD, 3mm-connector, 2UC3: 2-unit cantilever FPD, 3mm-connector, 1UC4: 1-unit-cantilever FPD, 4mm-connector, 2UC4: 2-unit cantilever FPD, 4mm-connector, 1UC5: 1-unit cantilever FPD, 5mm-connector, 2UC5: 2-unit cantilever FPD, 5mm-connector, colored measurement bar (MPa).

The conventional connectors (3mm) proximal to the retaining abutments in the models 1UC-3 and 2UC-3 present the higher stress values compared to the same connectors of all the models investigated, while the stress in the 3mm-connector distal to the retaining abutment of the 2-unit cantilever restoration approaches the yield strength of the material used. These observations are also justified by the results published on clinical studies, reporting on the success rate of extensive restorations on severely reduced periodontal tissues, made by general practitioners, not previewing the special dimensioning of the connectors. A considerable increase of technical failure rate is reported, [134, 34, 36, 91] rising up to 41% in a 2-year period. [134]

On the contrary, clinical investigations, which were carried on by experts and emphasized on the designing of the restorations, demonstrated considerably less technical failures compared to published studies, which did not incorporate special design demands. [103, 110]More specific, Nyman and Lindhe (1979) and Lundgren (1987), investigating the long–

term prognosis of cantilever cross-arch FPDs on minimal periodontal tissues, including special modifications of the prosthesis design, reported 0-8% technical complications within a 5- to 8- year period. [103, 110] These design modifications were referring to the dimensioning of connectors, which involved the increase of the VD and HD of the connectors proximal to the retaining abutment up to 6mm and 5mm, respectively. [105, 107] In the present study, the variable investigation involved the increase of the connectors VD up to 5mm, while the HD remained at 2,5mm. This was selected due to the limitation of the teeth size existing in the specific human population group and the use of the restorative materials.

The VD increase of the connector distal to the retaining abutment proved to be quite beneficial for the stress field developed within. Particularly, the gradual increase from 3mm to 5mm resulted in a stress decrease of about 50%. This is particularly significant for the 2-unit cantilever restorations, where their conventional connector distal to the retaining abutment presented stress values that were close to the critical yield strength value of its material. This determined the VD at the connection of the cantilever segment distal to the retaining abutment as a serious prognostic factor for the longevity of the 1- and 2- unit cantilever FPDs, even if it is not accompanied by simultaneous increase of the HD.

The fact, that the increase of the VD causes a decrease of the maximum von Mises stress appearing on the connector distal to the retaining abutment, is explained from the technical theory of bending. In particular and without loss of generality, the dominating mechanical behaviour of the retaining abutment under the application of occlusal forces is bending as shown in Figure 7, A and B.In more details, the retaining abutment and the cantilever unit are shown in Figure 7, A and B, where in Figure 7, A the connector VD for a section AA' located at x_A is h1 and in Figure 7, B the connector VD in the corresponding section AA' is h2>h1. However, it is well-known from the technical theory of bending that the maximum bending stress at any cross-section AA' is equal to:

$$\sigma_{\max} = \left(\frac{M}{I_{yy}} \right) z_{\max} \qquad \text{(eq.1)}$$

where

$$I_{yy} = \left(\frac{1}{12} \right) bh^3 \text{ and } z_{\max} = h \qquad \text{(eq.2)}$$

Introducing (eq.2) in (eq.1), and after basic manipulations, it yields:

$$\sigma_{\max} = \left(\frac{12M}{b} \right) \left(\frac{1}{h^2} \right) \qquad \text{(eq.3)}$$

where M is the bending moment at the cross-section under consideration. For a specific configuration, the quantities M and b are constant. Therefore, from eq.3, it yields that as h, that is the VD, increases, $\sigma_{\max}$ decreases. However, since bending is the predominant mechanical behaviour in the case under examination, the appearing maximum von Mises

stress, predominantly affected by σ_{max}, decreases as well. These observations comply with the aforementioned clinical studies, [103,110] linking the long-term functionality of the cross-arch FPDs to the connectors' dimensions and proving the high impact of VD. However, it must be noticed that despite the considerable stress decrease, the measured values were still the higher compared to the other connectors among the splinted teeth.

As far as the connector mesial to the retaining abutment is concerned, its VD increase had not a similar intense impact to the peak stress relief. In the case of the 2-unit cantilever restoration a small gradual relief of the peak stress value was measured, whereas in the 1-unit case the same value presented a fluctuated pattern. However it is clear (Figure 5, A through F) that the connector are benefited in terms of a gradually relieved stress distribution across its geometry. Either way the changes were relatively small, leading to the assumption of a low impact of VD on the peak stress for this connector, as well as for all the other mesial connectors. This can be explained from the fact that the application of the occlusal forces, in combination with the support provided by the PDL to each tooth, makes the teeth/connectors assembly behave quite like as a cantilever which is fixed along a significant part of its length (in opposition to a cantilever with a fixed point-end). This part extends in a zone from the central incisor to the second premolar (retaining abutment). As a result, increasing the VD does not affect the stress field of the connectors between the teeth of the aforementioned zone, within which the relative teeth displacements remain the same.

On the abutment level, the stress values obtained for the teeth #41-44 are not significant, while a considerable stress concentration is observed at the distal aspect of the #45 for all the models. Increase of the stress values at almost 100% is observed after the addition of the second cantilever for all the models, independently of the size of the connector proximal to the retaining abutment. The previous are in accordance with the FEA studies of Yang (1996) and Wang (1998). [119, 120]The increase of the connectors' vertical dimension does not result in significant changes for the end-abutment tooth in the 1 unit-cantilever restorations. Though, increase of the connectors' vertical dimension at 5mm for the 2-unit cantilever restoration results in a stress increase of almost 300% compared to the values obtained for the same connectors in the 2-unit cantilever restorations. On the abutment level, in a meta-analysis study, the reported incidence of fracture of abutment teeth in cantilever FPDs was 2.9 and 2.6% of the FPDs were lost as a result of the abutment tooth fracture after an observation period of 10 years. [103]Clinical studies with special biomechanical demands as far as concern the dimensioning of the metal framework report low incidence of tooth fracture, though no data concerning the loss of the tooth vitality are available, which may be related with the high stress concentration. [33, 110, 35]

On the periodontal ligaments level, the differences of the stress magnitude among the teeth #41-44 are minor. The stress is concentrated on the periodontal ligament of the #45. These observations are the same as Wylie and Caputo (1991) and Wang (1998). [40,42] In the present study, there is a 100% stress increase in the periodontal ligament of the terminal abutment after the addition of the first cantilever unit, which is not changed by the increase of the connectors' vertical dimension. The addition of the second cantilever unit results a stress increase of approximately 1MPa, compared to the 1-unit cantilever restoration. Though, the stress increase is getting higher when the connectors' vertical dimension reaches the 5mm, for the 2-unit cantilever restorations. The above observations are not in accordance with the FEA study o Yang (1996), who reports marked stress increase on the periodontal ligament after the

addition of the second cantilever, but justifies that the limitations for the application of cross-arch FPDs in the perioprosthetic patient are related to the technical and biomechanical problems involved rather than to the remaining periodontium to support the restoration successfully. [119]

Conclusion

The biomechanical response of the biological tissues to the extrinsic mechanical stimulations in the perioprosthetic patient is the result of a dynamic adaptive equilibrium of a hierarchically structured network, the nodes of which constitute, in a macroscopic level, the severely reduced periodontium and the dental tissues. The model of the network is the theoretical approach of describing the capability of the system to transmit, store and dissipate energy through its components and, thus, the medium to foresee the properties of a single nodal malfunction, which can start a global failure cascading in the system. Cascades follow a threshold rule meaning that a node will change state only if exceed a given threshold. Insofar as in advanced periodontal cases, the periodontium's integrity is severely compromised and the dental tissues' function is non-physiological, the failure cascade initiation is considered to be triggered with non expected distribution by different and lower threshold values. This means that, in the perioprosthetic patient, the system experiences differently the mechanical stimulation and a functional loading case may exert progressively increased tooth mobility as an indicator of progressive periodontal tissue collapse. Thus, the prosthetic treatment of the cantilever cross-arch fixed partial denture applied on the perioprosthetic patient aims at the re-establishment of a stable system capable of capturing the collapse initiation along with the safeguard of its own restorative integrity. The balance between, as indicated by clinical and auxiliary theoretical studies, seems to be related to the restoration's metal framework design and dimensioning, while, in case of balance inclination, the system suffers either from fracture on the weakest areas of the metal framework's connectors or from the collapse of the weakest distalmost abutment in terms of periodontal or dental complications.

References

[1] Kaÿser AF. Shortened dental arches and oral function. J Oral Rehabil 1981;8:457–62.

[2] Stahl SS: Marginal lesion. In: Goldman HM, Cohen DW (eds): Periodontal Therapy, ed 4. St. Louis: Mosby, 1968, pp 120.

[3] Amsterdam M: Periodontal prosthesis: Twenty-five years in retrospect. Alpha Omegan, December 1974.

[4] Kay HB, Keough BE, Rosenberg MM, Holt RL: Presurgical prosthetic diagnosis and management. In: Rosenberg MM (eds): Periodontal and prosthetic management for advanced cases.Quint Pub Co., Inc. 1988 pp. 61.

[5] Bragger U, Hakanson D, Lang NP. Progression of periodontal disease in patients with mild to moderate adult periodontitis. *J. Clin. Periodontol.* 1992; 9:659-67.

[6] Michalowicz BS. Genetic factors for periodontal diseases. *Comp. Contin. Educ. Dent*. 1994;25:1036-50.

[7] Mohammad AR, Jones JD, Brunsvold MA. Osteoporosis periodontal disease. A review. *Calif. Dent. Assoc. J*. 1994;22:69-75.

[8] Beck JD, Koch GG, Rozier G, Tudor GE. Prevelance and risk factors indicators for periodontal attachment loss in a population of older community-dwelling blacks and whites. *J. Periodontol*. 1990;61:521-8.

[9] Locker D, Leake JL. Risk indicators and risk markers for periodontal disease experience in older adults living independently in Ontario, Canada. *J. Dent. Res.* 1993; 72: 9-17.

[10] Grossi SG, Zambon JJ, Ho AW, Koch G, Machtei EE, Nordeyd OM, Genco RJ. Assessment of risk for periodontal disease (I). Risk indicators for attachment loss. *J. Periodontol.* 1994;65:260-7.

[11] Hart TC, Shapira L, Van Dykete. Neutrophil defects as risk factors for periodontal diseases. *J. Periodontol.* 1994;65:521-9.

[12] Machtei EE, Dunford R, Hausmann E, Grossi SG, Powell J, Cummins D, Zambon JJ, Genco RJ. Longitudinal study of prognostic factors in established periodontitis patients. *J. Clin. Periodontol*. 1997;24:102-9

[13] Fardal O, Johannessen AC, Linden GJ. Tooth loss during maintenance following periodontal treatment in a periodontal practice in Norway. *J. Clin. Periodontol.* 2004; 31: 550-55.

[14] Mc Guire MK. Prognosis versus actual outcome:Long-term survey of 100 treated periodontal patient under maintenance care. *J. Periodontol*. 1991;62:51-5.

[15] McGuire MK, Nunn M. Prognosis versus actual outcome.II.The effectiveness of clinical parameters in developing an accurate prognosis. *J. Periodontol.* 1996;67:658-65.

[16] McGuire MK, Munn M. Prognosis versus actual outcome.III.The effectiveness of clinical parameters in accurately predicting tooth survival. *J. Periodontol.* 1996;67:666-74.

[17] McGuire MK, Nunn M. Prognosis versus actual outcome.IV.The effectiveness of clinical parameters and IL-1 genotype in accurately predicting prognosis and tooth survival. *J. Periodontol.* 1999;70:49-56.

[18] McLeod DE, Lainson PA, Spivey JD. The predictability of periodontal treatment as measured by tooth loss : a retrospective study. *Quint .Int.* 1998;29:631-5.

[19] Hirschfeld L, Wasserman B. A long-term survey of tooth loss in 600 treated periodontal patients. *J. Periodontol.* 1978;49:225-37.

[20] Kaldhal W, Kalkwarf KL, Patil KD, Molvar MP, Dyer JK. Long term evaluation of periodontal therapy:II.Incidence of sites breaking down. *J. Periodontol.* 1996;67:103-8.

[21] McFall WT. Tooth loss in 100 treated patient with periodontal disease. *J. Periodontol.* 1982; 53:539-49.

[22] Lindhe J, Haffajee AD, Socransky SS. Progression of periodontal disease in adult subjects in the absence of periodontal therapy. *J. Clin. Periodontol*. 1983;10:433-42.

[23] Loe H, Anerud A, Boysen H, Morrison E. Natural history of periodontal disease in man. *J. Clin. Periodontol*. 1986;13:431-40.

[24] Wilson TG Jr, Glover ME, Malik AK, Schoen JA, Dorsett D. Tooth loss in maintenance patients in a private periodontal practice. *J. Periodontl.* 1987;58:231-5.

[25] Listgarten MA, Sullivan P, George C, Nitkin L, Rosenberg ES, Chilton NW, Rosenberg ES, Kramer AA. Coparative longitudinal study of 2 methods of scheduling maintenance visits:4-year data. *J. Clin. Periodontol*. 1989;16:105-15.

[26] Moser P, Hammerle CHF, Lang NP, Perrson R. Maintenance of periodontal attachment levels in prosthetically treated patients eith gingivitis or moderate chronic periodontitis 5-17 years post therapy. *J. Clin. Periodontol.* 2002;29:531-9.

[27] Konig J, Plagmann HC, Langefeld N, Kocher T. Retrospective comparison of clinical variables between compliant and non-compliant patients. *J. Clin. Periodontol*. 2001;28: 227-32.

[28] Lang NP, Tonetti MS. Periodontal risk assessment (PRA) for patients in supportive periodontal therapy. *Oral Health Prev. Dent*. 2003;1:7-16.

[29] Nieri M, Muzzi L, Cattabriga M, Rotundo R, Cairo F, Prato GPP. The prognostic value of several periodontal factors measured as radiographic bone level variation: a 10-year retrospective multilevel analysis of treated and maintained patients. *J. Periodontol.* 2002; 73: 1485-93.

[30] Leung WK, Ng DKC, Jin L, Corbet EF. Tooth loss in treated periodontitis patients responsible for their supportive care arrangements. *J. Clin. Periodontitis* 2006;33:265-75.

[31] Lundgren D, Nyman S, Heijl L, Carlsson GE. Functional analysis of fixed bridges on abutment teeth with reduced periodontal support. *J. Oral Rehabil.* 1975;2:105-16.

[32] Nyman S, Ericsson I. The capacity of reduced periodontal tissues to support fixed bridgework. *J. Clin. Periodontol.* 1982;9:409-14.

[33] Nyman S, Lindhe J. A longitudinal study of combined periodontal and prosthetic treatment of patients with advanced periodontal disease. *J. Periodontol*. 1979;50:163-69.

[34] Randow K, Glantz PO, Zoger B. Technical and some related clinical complications in extensive fixed prosthodontics. An epidemiological study of long-term clinical quality. *Acta Odontol. Scand*. 1986;44:241-55.

[35] Laurell L, Lundgren D, Falk H, Hugoson A. Long-term prognosis of extensive polyunit cantilevered fixed partial dentures. *J. Prosthet. Dent.* 1991;66:545-25.

[36] Yi SW, Carlsson GE, Ericsson I, Wennström JL. Long-term follow-up of cross-arch fixed partial dentures in patients with advanced periodontal destruction: evaluation of occlusion and subjective function. *J. Oral Rehabil*. 1996;23:186-96.

[37] Yi SW, Carlsson GE, Ericsson I. Prospective 3-year study of cross-arch fixed partial denture in patients with advanced periodontal disease. *J. Prosthet. Dent.* 2001;86:489-94.

[38] Hallmon HH. Occlusal trauma : Effect and impact on the periodontium. *Ann. Periodontol*. 1999;4:102-7.

[39] Brakatsoulas K, Koidis P. Trauma from occlusion and its relationship to periodontal disease. *Stoma* 1998;26:175-83.

[40] Lemmerman K. Rationale for stabilization. *J. Periodontol.* 1974;47:405-11.

[41] Davis SJ, Grey RJM, Lindhe JG, James JA. Occlusal consideration in periodontics. *Brit. Dent. J*. 2001;191:597-604.

[42] Hanamura H, Houston F, Rylander H, Carlsson GE, Haraldson T, Nyman S. Periodontal status and bruxism- a comparative study of patients with periodontal disease and occlusal parafunctions. *J. Periodontol.;*58:173-6.

[43] Wentz F, Jarabak J, Orban B. Experimental occlusal trauma imitating cuspal interferences. *J. Periodontol.* 1958;29:117-27.
[44] Stahl SS. The responses of the periodontium to combined gingival inflammation and occluso-functional stresses in four human surgical speciments. *Periodontic* 1968;6:14-22.
[45] Comar MD, Kollar JA, Gargiulo AW. Local irritation and occlusal trauma as co-factors in the periodontal disease process. *J. Periodontol.* 1969;40:193-200.
[46] Glickman I, Smullow JB. Alterations in the pathwae of gingival inflammation into underlying tissues induced by excessive occlusal forces. *J. Periodontol*. 1962;33:7-13.
[47] Cher ME. Changing concepts. The effects of occlusion on periodontitis. *Dent. Clin. North Am*. 1998;42:285-97.
[48] Waerhaug J. The infrabony pocket and its relationship to trauma from occlusion and subgingival plaque. *J. Periodontol.* 1979;50:355-65.
[49] Lindhe J, Ericsson I. Influence of trauma from occlusion on reduced but healthy periodontal tissues in dogs. *J. Clin. Periodontol.* 1976;3:110-22.
[50] Perrier M, Polson A. The effect of progressive and increasing tooth ypermobility on reduced but healthy periodontal supporting tissues. *J. Periodontol.* 1982;53:152-7.
[51] Polson AN, Zander HA. The effect of periodontal trauma upon intrabony pockets. *J. Periodontol*. 1983;54:586-92.
[52] Ericsson I, Lindhe J. Effect of long standing jiggling on experimental periodontitis in the beagle dog. *J. Clin. Periodontol*. 1982;9:497-503.
[53] Neirude AM, Ericsson I, Lindhe J. Probing pocket depth at mobile/non-mobile teeth. *J. Clin. Periodontol.* 1992;19:754-9.
[54] Kvinnsland S,Kristiansen AB, Kvinnsland I, Heyeraas KJ. Effect of experimental traumatic occlusion on periodontal and pulpal blood flow. *Acta Odontol. Scand.* 1992; 50: 211-9.
[55] Philstrom BL, Anderson KA, Aeppli D, Schaffer EM. Association between signs of trauma from occlusion and periodontitis. *J. Periodontol.* 1986;57:1-6.
[56] Jin LJ, Cao CF. Clinical diagnosis of trauma from occlusion and its relation with severity of periodontitis. *J. Clin. Periodontol.* 1992;19:92-7.
[57] Serio FG, Hawley CE. Periodontal trauma and mobility. Diagnosis and treatment planning. *Dent. Clin. North Am*. 1999;43:37-44.
[58] Ramfjord SP, Major MA. Significance of occlusion in the etiology and treatment of early, moderate and advanced periodontitis. *J. Periodontol.* 1981;52:511-7.
[59] Polson AM. Trauma and progression of marginal periodontitis in squirrel monkeys.:III. Adaptation of interproximal alveolar bone to repetitive injury. *J. Periodont. Res.* 1976a;11:279-89.
[60] Glickman I. Inflammation and trauma from occlusion; co-destructive factors in chronic periodontal disease. *J. Periodontol.* 1963;34:5-10.
[61] Carranza FA, Simes RJ, Mayo J, Carbini RL. Histometric evaluation of periodontal bone loss in rats.I. The effect of marginal irritation, systemic irradiation and trauma from occlusion. *J. Periodont. Res.* 1971;6:65-72.
[62] Kenney DB. A histopathologic study of incisal dysfunction and gingival inflammation in the rhesus monkey. *J. Periodontol.* 1971;42:3-7.
[63] Lindhe J, Svanberg G. Influence of trauma from occlusion on progression of experimental periodontitis in the beagle dog. *J. Clin. Periodontol*. 1974;1:3-14.

[64] Nyman SS, Lindhe J, Ericsson I. The effect of progressive tooth mobility on destructive periodontitis in the dog. *J. Clin. Periodontol.* 1978;5:213-25.
[65] Meitner S. Co-destructive factors of marginal periodontitis and repetitive mechanical injury. *J. Dent. Res.* 1975;54:C78-C85.
[66] Kantor M, Polson AM, Zander HA. Alveolar bone regeneration after removal of inflammatory and traumatic factors. *J. Periodontol.* 1976;47:687-95.
[67] Polson AM, Meitner SW, Zander HA. Trauma and progression of marginal periodontitis in squirrel monkeys. *J. Per. Res*. 1976b;11:290-7.
[68] Polson AM, Adams RA, Zander HA. Osseous repair in the presence of active tooth ypermobility. *J. Clin. Periodontol.* 1983;10:370-9.
[69] Svanberg GK, King JJ, Gibbs CH. Occlusal considerations in periodontology. *Periodontol.* 2000 1995;9:106-17.
[70] Shefter G, McFall W. Occlusal relations and periodontal status in human adults. *J. Periodontol.* 1984;55:368-74.
[71] Yodelis R, Mann WV. The prevelance and possible role of nonworking contacts in periodontal disease. *Periodontics* 1965;3:219-23.
[72] Houston F, Hanamura H, Carlsson GE. Mandibular disfunction and periodontitis. A comparative study of patients with periodontal disease and occlusal parafunctions. *Acta Odontol. Scand*. 1987;45:239-46.
[73] Budtz-Jorgensen E. Bruxism and trauma from occlusion. An experimental model in Macaca monkeys. *J. Clin. Periodontol*. 1980;7:149-62.
[74] Hakkarainen K. Relative influence of scaling and root planning and occlusal adjustment on sulcular fluid flow. *J. Periodontol.* 1986;57:684-91.
[75] Proceedings of the world workshop in clinical periodontics. Chicago:The americal academy of periodontology. 1989:III-15-22.
[76] Nunn ME, Harrel. The effect of occlusal discrepancies on periodontitis. I. Relationship of initial occlusal discreapancies to initial clinical parameters. *J. Periodontol.* 2001;72:484-94.
[77] Harrel S, Nunn ME. The effect of occlusal discrepancies on periodontitis.II. Relationship of occlusal treatment to the progression of periodontal disease. *J. Periodontol.* 2001;72:494-505.
[78] Nyman S, Lang N. Tooth mobility and biological rational for splinting teeth. *Periodontol*. 2000 1995;4:15-22.
[79] Siegel SC, Driscoll CF, Feldman S. Tooth stabilization and splinting before and after periodontal therapy with fixed partial dentures. *Dent. Clin. North Am*. 1999;43:45-76.
[80] Ferencz J. Splinting. *Dent. Clin. North Am.* 1987;31:383-93.
[81] Wylie RS, Caputo AA. Fixed cantilever splints on teeth with normal and reduced periodontal support. *J. Prosthet. Dent*. 1991; 66:737-42.
[82] Renggli HH, Schweiser H. Splinting of teeth with removable bridges. *J. Clin. Periodontol.* 1974;1:43-6.
[83] Renggli HH, Allet B, Spanauf AJ. Splinting of teeth with fixed bridges. *J. Oral Reh.* 1984;6:535-7.
[84] Kanno T, Carlsson GE. A review of the shortened dental arch concept focusing on the work by the Käyser/Nijmegen group. *J. Oral Rehabil.* 2006;33:850-62.

[85] Hämmerle CH, Ungerer MC, Fantoni PC, Brägger U, Bürgin W, Lang NP. Long-term analysis of biologic and technical aspects of fixed partial dentures with cantilevers. *Int. J. Prosthodont.* 2000;13:409-15.

[86] Isidor F, Budtz-Jørgensen E. Periodontal conditions following treatment with distally extending cantilever bridges or removable partial dentures in elderly patients. A 5-year study. *J. Periodontol.* 1990;61:21-6.

[87] Budtz-Jørgensen E, Isidor F. A 5-year longitudinal study of cantilevered fixed partial dentures compared with removable partial dentures in a geriatric population. *J. Prosthet. Dent.* 1990;64:42-7.

[88] Misch CE, Steingnga C, Barboza E, Misch-Dietsh F, Cianciola LJ, Kazor C. Short dental implants in posterior partial edentulism: a multicenter retrospective 6-year caseseries study. *J. Periodontol.* 2006;77:1340-7.

[89] Ante IH. The fundamental principles of abutments. Thesis. Mich. State Dent. Soc. Bulletin 1926;8:14-23.

[90] Glantz PO, Nyman S. Technical and biophysical aspects of fixed partial dentures for patients with reduced periodontal support. *J. Prosthet. Dent*. 1982;47:47-51.

[91] Karlsson S. Failures and length of service in fixed prosthodontics after long-term of function. *Swed. Dent. J*. 1989;13:185-92.

[92] Sorensen JA, Martinnoff JT. Endodontically treated teeth as abutments. *J. Prosthet. Dent.* 1985;53:631-6.

[93] Bergenholtz G, Nyman S. Endotontic complications following periodontal and prosthetic treatment of patients with advanced periodontal disease. *J. Periodotnol.* 1984;55:63-8.

[94] Strub JR, Linter H, Marinello CP. Rehabilitation of partially edentulous patients using cantilever bridges: A retrospective study. *Int. J. Periodont. Rest. Dent.* 1989;9:365-75.

[95] Landolt A, Lang NP. Erfolg und Missefolg bei extensionbrucken. Schweiz Monatssch Zahmed 1988;98:239-44.

[96] Owall BE, Almfeldt I, Helbo M. Twenty-year experience with 12-unit fixed partial dentures supported by two abutments. *Int. J. Prosthodont.* 1991:4:24-9.

[97] Laurell L, Lundgren D. Interfering occlusal contacts and distribution of chewing and biting forces in dentitions with fixed cantilever prostheses. *J. Prosthet. Dent.* 1987; 58: 197-203.

[98] Laurell L, Lundgren D, Falk H, Hugoson A. Long-term prognosis of extensive polyunit cantilevered fixed partial dentures. *J. Prosthet. Dent.* 1991;66:545-52.

[99] Lundgren D. Prosthetic reconstruction of dentitions seriously compromised by periodontal disease. *J. Clin. Periodontol.* 1991;18:390-5.

[100] Laurell L, Lundgren D. Distribution of occlusal forces along unilateral posterior two-unit cantilever segment in cross-arch fixed partial dentures. *J. Prosthet. Dent.* 1988; 60: 106-12.

[101] Laurell L, Lundgren D. Influenece of occlusion on posterior cantilevers. *J. Prosthet. Dent.* 1992;67:645-52.

[102] Falk H, Laurell L, Lundgren D. Occlusal force pattern in dentitions with mandibular implant-supported cantilever prostheses occluding with complete dentures. *Int.J.Oral.Maxillofac.Impl.* 1989;4:55-62.

[103] Tan K, Pjetursson BE, Lang NP, Chan ES. A systematic review of the survival and complication rates of fixed partial dentures (FPDs) after an observation period of at least 5 years. *Clin. Oral Implants Res.* 2004;15:654-66.

[104] Pjetursson BE, Tan K, Lang NP, Brägger U,Egger M, Zwahlen M. A systematic review of the survival and complication rates of fixed partial dentures (FPDs) after an observation period of at least 5 years. *Clin. Oral Implants Res*. 2004;15:667-76.

[105] Lundgren D. Prosthetic reconstruction of dentitions seriously compromised by periodontal disease. *J. Clin. Periodontol*. 1991;18:390-5.

[106] Karlsson S. Failures and length of service in fixed prosthodontics after long-term function. Alongitudinal study. *Swed. Dent. J*. 1989;13:185-92.

[107] Kourkouta S, Hemmings KW, Laurell L. Restoration of periodontally compromised dentitions using cross-arch bridges. Principles of perio-prosthetic patient management. *Br. Dent. J*. 2007;203:189-95.

[108] Manda M, Galanis C, Georgiopoulos V, Provatidis C, Koidis P. Effect of severely reduced bone support on the stress field developed within the connectors of three types of cross-arch fixed partial dentures. *J. Prosthet. Dent.* 2009;101:54-65.

[109] Erhardson S, Carlsson J, Wictorin L. Fracture mechanics design of dental soldered joints. *Swed. Dent. J. Suppl*. 1980;1-62.

[110] Lundgren D, Laurell L. Occlusal force pattern during chewing and biting in dentitions restored with fixed bridges of cross-arch extension. II. Unilateral posterior two-unit cantilevers. *J. Oral Rehabil.* 1986;13:191-203.

[111] Hanihara T, Ishida H. Metric dental variation of major human populations. *Am. J. Phys. Anthropol.* 2005;128:287-98.

[112] Craig RG. Advances in biomaterials from 1957 to 1997. *J. Oral Rehabil.* 1999;26:841-6.

[113] Oh WS, Anusavice KJ. Effect of connector design on the fracture resistance of all-ceramic fixed partial dentures. *J. Prosthet. Dent.* 2002;87:536-42.

[114] Oh W, Götzen N, Anusavice KJ. Influence of connector design on fracture probability of ceramic fixed-partial dentures. *J. Dent. Res.*2002;81:623-7.

[115] Rodriguez AM, Aquilino SA, Lund PS. Cantilever and implant biomechanics: a review of the literature. Part 1. *J. Prosthodont*. 1994;3:41-6.

[116] Schweitzer JM, Schweitzer RD, Schweitzer J. Free-end pontics used on fixed partial dentures. *J. Prosthet Dent.* 1968;20:120-38.

[117] Glantz PO, Nyman S, Strandman E, Randow K. On functional strain in fixed manibular reconstructions. II. An in vivo study. *Acta Odontol. Scand*. 1984;42:269-76.

[118] Awadalla HA, Azarbal M, Ismail YH, el-Ibiari W. Three dimensional finite element stress analysis of a cantilevered fixed partial enture. *J. Prosthet Dent.* 1992;68:243-8.

[119] Yang HS, Chung HJ, Park YJ. Stress analysis of a cantilevered fixed partial denture with normal and reduced bone support. *J. Prosthet Dent*. 1996;76:424-30.

[120] Wang CH, Lee HE, Wang CC, Chang HP. Methods to improve a periodontally involved terminal abutment of a cantilevered fixed partial denture--a finite element stress analysis. *J. Oral Rehabil.* 1998;25:253-7.

[121] Yang HS, Lang LA, Felton DA. Finite element stress analysis on the effect of splinting in fixed partial dentures. *J. Prosthet Dent.* 1999;81:721-8.

[122] Eraslan O, Sevimay M, Usumez A, Eskitascioglu G. Effects of cantilever design and material on stress distribution in fixed partial dentures--a finite element analysis. *J. Oral Rehabil*. 2005;32:273-8.
[123] Augereau D, Pierrisnard L, Barquins M. Relevance of the finite element method to optimize fixed partial denture design. Part I. Influence of the size of the connector on the magnitude of strain. *Clin. Oral Investig.*1998;2:36-9.
[124] Motta AB, Pereira LC, da Cunha AR, Duda FP. The influence of the loading mode on the stress distribution on the connector region of metal-ceramic and all-ceramic fixed partial denture. *Artif. Organs.* 2008;23:283-91.
[125] Taskonak B, Yan J, Mecholsky JJ Jr, Sertgöz A, Koçak A. Fractographic analyses of zirconiabased fixed partial dentures. *Dent. Mater.* 2008;24:1077-82.
[126] Kamposiora P, Papavasiliou G, Bayne SC, Felton DA. Stress concentration in all-ceramic posterior fixed partial dentures. *Quintessence Int.* 1996;27:701-6.
[127] Marinescu R, Daegling DJ, Rapoff RJ. Finite element modeling of the anthropoid mandible: the effects of altered boundary conditions. *Anat. Rec. A Discov. Mol. Cell Evol. Biol.* 2005;283;300-9.
[128] Romeed SA, Fok SL, Wilson NH. A comparison of a 2D and 3D finite element analysis of a restored tooth. *J. Oral Rehabil*. 2006;33:209-15
[129] Al-Sukhun J, Lindqvist C, Helenius M. Development of a three-dimensional finite element model of a human mandible containing endosseous dental implants. II. Variables affecting the predictive behavior of a finite element model of a human mandible. *J. Biomed. Mater. Res.* A 2007;80:247-56.
[130] Budynas RG, Nisbett JK. Shigley's mechanical engineering design. New York: McGraw- Hill; 2006. p. 28.
[131] Kythe PK, Wei D. An introduction to linear and nonlinear finite element analysis: a computational approach. Boston: Birkhäuser; 2004. p. 75.
[132] McLean JW. Construction of Metal-Ceramic Bridgework. In: McLean JW (eds). The science and art of dental ceramics. Volume II: Bridge design and laboratory procedures in dental ceramics. Chicago: Quintessence; 1980. p. 331.
[133] Manda M, Galanis C, Georgiopoulos V, Provatidis C, Koidis P. Effect of varying the vertical dimension of connectors of cantilever cross-arch fixed partial dentures in patients with severely reduced osseous support. *J. Prosthet. Dent.2010;103:91-100*.
[134] Owall B, Cromström R. First two-year complications of fixed partial dentures, eight units or more. Swedish Guarantee Insurance claims. *Acta Odontol. Scand*. 2000;58:72-6.
[135] Lundgren D, Laurell L, Falk H, Hugoson A. Long term prognosis of extensive fixed bridges with posterior two-unit cantilevers. A retrospective study. NOF, The Scandinavian Dicision of IADR 1987, special issue report 22

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Chapter IV

Biomarkers of Periodontal Disease: Past, Present and Future Challenges

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Abstract

Periodontal disease is a chronic bacterial infection characterised by persistent inflammation, connective tissue breakdown and alveolar bone destruction. The chronic inflammation associated with periodontal disease represents the host response to bacterial plaque, mediated by the environment in which the response occurs. Periodontitis is both site-specific and episodic in nature and thus biomarker development could prove invaluable in identifying sites with active disease, predicting sites that may develop disease, monitoring response to therapy or identifying patients with enhanced disease susceptibility.

In periodontal disease gingival crevicular fluid (GCF) flows from the gingival microcirculation into the periodontal pockets and the volume increases in proportion to the severity of the local inflammatory process.The study of GCF samples, from defined sites of chronic periodontal inflammation, allows non-invasive access to an inflammatory exudate that could be used for biomarker discovery.GCF contains proteins synthesised and secreted in the inflamed gingival tissues and carried by the GCF to the gingival crevice/pocket. Here, they are augmented by proteins released from bacteria and host cells, particularly polymorphonuclear leukocytes (PMNs), present in the periodontal pocket. The constituents of GCF are therefore derived from a number of sources including microbial plaque, host inflammatory cells, serum and tissue breakdown products.Saliva has also been studied in the search for biomarkers of periodontal disease.Saliva is a more complex fluid, comprising glandular secretions, components of GCF, components of serum and also particles (including bacteria) from a variety of oral

and airway sources.Although saliva has the advantage of being easily collected, its biochemical complexity may hinder detection of biomarkers specific for periodontal disease. Furthermore the fact that saliva bathes the whole mouth negates the use of salivary biomarkers for site-specific identification or monitoring of periodontal disease.

Despite an impressive list of possibilities, biomarkers have yet to reach routine clinical use as reasonable predictors of periodontal status. This chapter reviews the analysis of GCF and saliva for monitoring periodontal health and disease. Potentially important biomarkers of disease in both GCF and saliva are highlighted and their merits are described in further detail. Putative biomarkers from both host and bacterial sources are considered and the use of multiple biomarkers is discussed. Following the technological revolution in both genomic and proteomic analysis over the last decade it is tempting to speculate that the next decade could bring much waited progress in the field of biomarker identification and application in the field of periodontal disease.

Introduction

Periodontal Diseases

Plaque-induced periodontal diseases have traditionally been divided into two general categories based on whether attachment loss has occurred: gingivitis and periodontitis. Gingivitis is the presence of gingival inflammation without loss of connective tissue attachment whereas in periodontitis there is or has been pathological detachment of collagen fibres from cementum and apical migration of the junctional epithelium (Anon, 2003). Gingival and periodontal inflammation represents the host response to bacterial plaque, mediated by the environment in which the response occurs (Genco, 1990). The supporting tissues of the teeth, including the periodontal ligament and alveolar bone, are destroyed in periodontitis and the end point is invariably the same, tooth loss. In the local lesion of periodontitis, as tissue damage progresses, gaps are formed between the gum and the root surfaces of the teeth referred to as periodontal pockets. In severe disease these periodontal pockets are several mm deep and are lined by ulcerated epithelium covering inflamed connective tissue. In periodontitis gingival crevicular fluid (GCF) flows from the gingival microcirculation into the periodontal pockets and the volume increases in proportion to the severity of the local inflammatory process.Factors controlling the progression of gingivitis to periodontitis remain to be fully elucidated and as a result key questions about the pathogenesis of the disease remain unanswered. Identification of a biomarker to characterise the transition between gingivitis and periodontitis would be a major discovery in terms of (1) identifying gingivitis cases at risk of progressing to periodontitis, (2) monitoring periodontal disease progression and (3) supporting appropriate therapeutic intervention.

Biomarkers

Biomarkers of disease should be objective measurements that act as indicators of normal biological processes, pathogenic processes or pharmacological responses to therapeutic intervention. There are at least three important technical attributes of a good biomarker: (1) presence in a peripheral body fluid (e.g., blood, saliva, GCF); (2) ease of detection or

quantification in robust and affordable assays; and (3) associated specifically with damage of a particular tissue, preferably in a quantifiable manner. Properties of the diagnostic test in which biomarkers are employed such as specificity and sensitivity are helpful in evaluating its usefulness in clinical practice. Very simply the relationship between the result of the test and the clinical diagnosis can be presented in a two by two table (Figure 1). The ideal test should have a sensitivity and specificity of 1, however no such test is currently available in medicine or dentistry. The positive predictive value is also important as it reflects the probability that a positive test reflects the underlying condition, however its value depends on the prevalence of the disease which may vary. The negative predictive value on the other hand is the portion of patients with negative test results who are correctly diagnosed.

Improving on Current Diagnostic Markers

Novel biomarkers of disease are generally measured against a 'gold standard'. No such gold standard exists for periodontal diseases as traditional biomarkers like bleeding on probing (BOP) are associated with many false positives and are not readily quantifiable. However, the absence of BOP is generally considered as an accurate negative predictor of disease (Lang et al., 1990). In the search for novel biomarkers comparisons must be made with current measures of disease. Conventional periodontal probes are used routinely to obtain both probing depth (PD; distance from the gingival margin to the base of the probable crevice) and clinical attachment loss (CAL; distance from the cementoenamel junction to the base of the probable crevice). PDs can be recorded rapidly and provide an overall assessment of the depth of periodontal pockets whereas CAL assessments are more difficult to measure accurately, but give a better overall estimate of the amount of damage to the periodontium. The crudeness of measurements to the nearest mm to assess attachment loss means that the extent of disease is not determined by a reliable measure and thus disease progression may occur, but not to a large enough extent to be measurable routinely. Electronic probeshave better resolution but are not in routine clinical use (Jeffcoat et al., 1986).

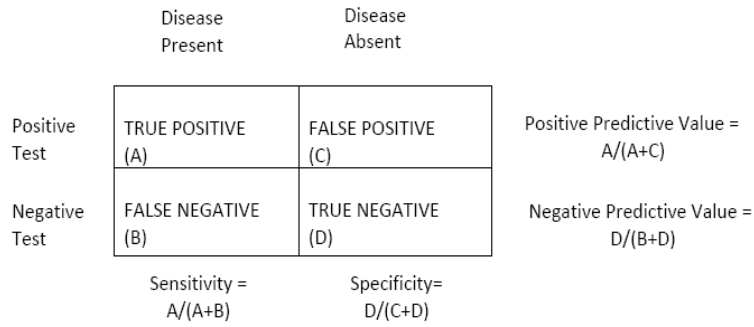

Figure 1. Decision matrix for diagnostic and prognostic tests.

The threshold for attachment loss must therefore be considered carefully if it is to be used as a gold standard for biomarker validation. Studies measuring 1 mm, 2 mm or 3 mm attachment losses over a defined period of time have been reported and those studies with large thresholds have a lower likelihood of false positives (and a higher likelihood of false

negatives). Disagreement between the attachment loss measured and the biomarker may therefore be a true reflection of better positive prediction by the latter. The majority of studies choose a CAL of 2 mm, with radiographic bone loss employed as a complementary method to diagnose disease progression. Despite inherent difficulties in defining the effectiveness of a novel biomarker against crude clinical measurements of disease there are currently few alternatives.

Biomarkers in GCF or Saliva?

The study of GCF samples, from defined sites of chronic periodontal inflammation, allows non-invasive access to an inflammatory exudate that can be used to improve our understanding of the inflammatory process. The protein concentration of GCF at periodontally healthy sites (22 mg/ml) is similar to that of physiological extravascular fluids (Curtis et al., 1988). By contrast, GCF from inflamed sites has a protein concentration in the region of 70 mg/ml and is considered to be a classical inflammatory exudate (Bickel et al., 1985). The cellular components of GCF are 70-80% granulocytes, 10-20% monocytes/ macrophages, 5% mast cells and 5% T lymphocytes. The fluid exudate contains proteins synthesised and secreted in the inflamed gingiva and carried by the GCF to the gingival crevice/pocket (Lamster, 1997). Here, they are augmented by proteins released from bacteria and host cells, particularly polymorphonuclear leukocytes (PMNs), present in the periodontal pocket. The constituents of GCF are therefore derived from a number of sources including microbial plaque, host inflammatory cells, serum and tissue breakdown (Curtis et al., 1989). The rationale behind the analysis of crevicular fluid is that inflammatory mediators present in GCF will reflect events taking place both within the connective tissue and in the gingival crevice/pocket. Despite the minute quantities of fluid that can be recovered from single gingival crevices or periodontal pockets (Linden et al., 1997), the majority of investigations on biomarkers of periodontitishave focused on GCF.

In periodontal disease individual sites undergo periods of active tissue destruction followed by periods of quiescence or repair. The site specific nature of the disease therefore points to the use of individual GCF samples for analysis since it is only by sampling at individual sites that a site specific prognosis can be evaluated. Specific markers of disease activity are more likely to be present at higher concentration in the micro-environment of the gingival crevice. However the collection of GCF, although non-invasive, requires training and expertise, and the technique should be perfected so as not to allow GCF sample contamination with saliva or blood. As a result of some of the inherent difficulties with sampling and analysis of minute quantities of GCF, researchers have looked to saliva as an alternative oral fluid for biomarker discovery. In some ways this can be regarded as an advantage as salivary analytes may offer a way of assessing subject-level (as opposed to site-level) risk or status. Furthermore, collection of whole saliva is easy, non-invasive and rapid, requiring no special equipment or expertise.There are, however, additional challenges in using saliva for diagnostic purposes. Whole or mixed saliva is a complex fluid mixture derived from the major and minor salivary glands, containing contributions from GCF, oral bacteria, cells and other sources that make identification of the exact site of disease activity difficult.A specific biomarker of periodontal disease is perhaps more likely to be present in higher levels in GCF and may not be measurable robustly in saliva. Furthermore, heightened

periodontal disease activity in a limited number of sites could be underestimated by a dilution effect in saliva. In addition, salivary flow rate varies between individuals or as a result of medications and could affect biomarker concentration in saliva.

Biomarkers Associated with Periodontal Disease Pathogenesis

Periodontal diseases reflect a spectrum of disease activity from mild gingivitis through to chronic destructive periodontitis.Gingivitis is a reversible inflammatory condition and does not result in the permanent destruction of the periodontal ligament or alveolar bone.Inflammatory markers associated with gingivitis would therefore not predict reliably the likelihood of a patient developing periodontal disease. Of the numerous constituents in GCF, however, the vast majority constitute soft tissue inflammatory events, while only a few are regarded as specific biomarkers of alveolar bone destruction. At present under-diagnosis of periodontal disease results in low rates of appropriate therapy and a reliable biomarker would help redress this balance in favour of improved prognosis. Novel biomarkers should correctly identify current disease activity, predict sites vulnerable for future breakdown, and assess the response to periodontal interventions, ultimately leading to improved clinical management of periodontal patients. Since periodontitis is a multifactorial disease, measurement of multiple biomarkers may be advantageous.

It has long been recognised that PMNs are the major cells of the acute inflammatory response in mammalian tissue and the oral cavity is no exception (Wilton et al., 1976). Many of the potential biomarkers studied in GCF are derived from PMNs and as such are early markers of inflammation but may not be useful discriminators between gingival inflammation and periodontal disease. Several biomarkers of disease are now discussed in more detail, in order of the phase of the disease process in which they have a major role; initial inflammation, extracellular matrix (ECM) breakdown and boneturnover/destruction (Figure 2). It must be pointed out that many of the putative biomarkers describedbelow are functional in more than one phase of disease.

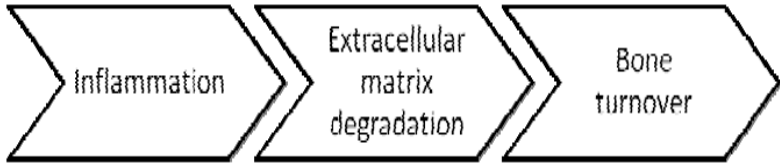

Figure 2. Simplified model of the progression of periodontal disease.

Biomarkers of Inflammation

During the initiation of an inflammatory response in the gingival and periodontal connective tissue numerous inflammatory mediators, such as prostaglandin E2 (PGE_2), interleukin (IL)-1β, IL-6, or tumour necrosis factor (TNF)-αare released from cells of the junctional epithelia, connective tissue fibroblasts, macrophages, and PMNs. Neuropeptides are also released from nerve fibres innervating the gingival and periodontal tissues and

represent an important component of the neurogenic inflammatory response (Lundy and Linden, 2004). Subsequent to the initial inflammatory response T and B cells emerge at the infection sites and secrete immunoglobulins (Kornman et al., 1997).

Prostaglandins

Prostaglandins are pro-inflammatory mediators synthesised from cell membrane phospholipids by the action of cyclo-oxygenases. Cross-sectional studies have shown that PGE_2 levels in GCF are elevated in diseased states and that PGE_2 levels are increased in gingivitis and periodontitis compared with health (Offenbacher et al., 1986). The levels of PGE2 were reported to correlate with severity of disease (Heasman et al., 1998; Tsai et al., 1998) and to discriminate between active and chronic disease.However analysis of PGE_2 is complex and has not been readily amenable to chair side diagnostics (McCauley et al., 2002).

Neuropeptides

Neuropeptides play important roles in neurogenic inflammation including vasodilation, plasma extravasation and recruitment of immune cells. However, a more extensive function for neuropeptides in the regulation of immune cell activity has also been proposed (Lundy and Linden, 2004). Nerve fibres innervating the periodontal tissues in humans are immunoreactive to several neuropeptides including substance P (SP), calcitonin gene-related peptide (CGRP), vasoactive intestinal polypeptide (VIP) and neuropeptide Y(NPY) (Luthman et al., 1988). Indeed SP (Linden et al., 1997) CGRP (Lundy et al., 1999), VIP (Linden et al., 2002) and NPY (Lundy et al., 2009) have all been detected in GCF. Although the presence of neuropeptides in GCF reflects their importance in gingival and periodontal inflammation it remains to be determined whether neuropeptides hold promise as biomarkers of disease.

Cytokines and Chemokines

Recent studies on the usefulness of cytokines as biomarkers of periodontitis have yielded conflicting results. Salivary levels of TNF-α were shown to be elevated in patients with clinical indicators of periodontitis (Frodge et al., 2008). However salivary levels of granulocyte–macrophage colony-stimulating factor, interleukin-1β, interleukin-2, interleukin-4, interleukin-5, interleukin-6, interleukin-8, interleukin-10, interferon-γ and tumor necrosis factor-α were later shown not to discriminate between periodontal health and disease (Teles et al., 2009a). In a checkerboard immunoblot analysis of inflammatory mediators in GCF, periodontitis subjects had statistically significantly higher mean levels of IL-1β and IL-8 compared to healthy subjects (Teles et al., 2009b). These findings are supported by a recent study in which the elevated odds of clinical periodontitis were associated with elevated levels of IL-1β in GCF (Fitzsimmons et al., 2010).Cytokine and chemokine measurements in GCF therefore appear to be more promising than salivary measurements in terms of biomarker potential.

Calprotectin

Neutrophils are the primary source of calprotectin, a 36-kDa protein composed of a dimeric complex of 8- and 14-kDa subunits. The 8 kDa subunit is also known as S100 A8, calgranulin A or macrophage migration inhibitory factor related protein-8 (MRP-8) and the 14 kDa subunit is also known asS100 A9, calgranulin B or MRP-14. MRP-8 has previously been identified (Lundy et al., 2000) and quantified (Lundy et al., 2001) in periodontitis.

Higher calprotectin levels have been reported in disease compared with health (Kido et al., 1999). It has been suggested that calprotectin improves resistance to *Porphyromonas gingivalis* by boosting the barrier protection and innate immune functions of the gingival epithelium (Nisapakultorn et al., 2001).

Immunoglobulins

The presence of antibodies to periodontal pathogens cannot be used to diagnose periodontitis but they can be employed to determine exposure to periodontopathic organisms. The detection of antibodies to particular periodontal pathogens *per se* appears to be a less useful measurement than the determination of antibodylevels (Ebersole and Holt, 1991). Immunological tests may be useful in identifying individuals who have the potential to develop periodontal disease or to monitor those who are currently responding to a periodontopathic infection. Using a checkerboard approach it has recently been shown that periodontitis patients have an enhanced nonspecific IgA response whereastheir specific IgA response, particularly to the periodontal pathogen *Aggregatebacter actinomycetemcomitans* was impaired (Bachrach et al., 2008). Antibody-based biomarker tests require much more development before their prognostic potential is realised fully.

Biomarkers of ECM Breakdown

An array of host derived enzymes, including neutral enzymes (elastase, cathepsin G), as well as hyrolysases (cathepsin B) and collagenases (matrix metalloproteinase (MMP)-8, MMP-9 and MMP-13) are stored and released from the granules of PMNs attracted to the gingival crevice. These enzymes have received substantial attention as potential biomarkers of ECM degradation, however some also have roles in bone turnover.

Elastase

Elastase is a principal proteolytic component of the neutrophil and functions to degrade the ECM. Elastase activity alone and in combination with other neutrophil enzymes in GCF has been shown to be related to gingival inflammation. Total enzyme activities have shown good diagnostic specificity and sensitivity as predictors of clinical parameters in cross-sectional studies (Eley and Cox, 2006), suggesting that GCF proteases, including elastase, may prove useful for determining periodontal condition.

Cathepsins

Cathepsins are a class of globular lysosomal proteases, most of which contain an active-site cysteine residue. Cathepsins function in general protein turnover in the lysosome, however several cathepsins have extracellular roles in degrading matrix components. As an enzyme belonging to the class of cysteine proteinases, cathepsin B functions in proteolysis of the ECM.GCF concentrations of cathepsin B have been found to be elevated in patients with periodontal disease compared with gingivitis (Kunimatsu et al., 1990). Cathepsin B has been shown to have potential in distinguishing periodontitis from gingivitis and in planning treatment and monitoring treatment outcomes (Loos and Tjoa 2005). In GCF, macrophages are thought to be the main source of cathepsin B (Kennett et al., 1997). Cathepsin K is an attractive candidate in terms of biomarker potential since it is expressed and secreted by

osteoclasts and has a key role in degrading bone matrix molecules. Cathepsin K levels have been shown to be increased in GCF from periodontitis patients (Mogi and Otogoto, 2007) however cathepsin-K was not associated significantly with clinical measurements of disease severity or inflammation indicating that more research on this important cathepsin is required to clarify its biomarker potential. GCF cathepsin G levels (determined by ELISA) showed significant correlation with measured clinical parameters of disease such as GCF volume, gingival index and probing depth, although cathepsin G activity in GCF did not correlate in the same way (Kunimatsu et al., 1995).

MMPs

Matrix metalloproteinases (MMPs) are zinc-dependent host endoproteinases derived predominantly from PMNs during acute stages of periodontal disease. MMPs are responsible for both tissue degradation and remodelling. As periodontal disease progresses gingival and periodontal ligament collagens are degraded by host cell-derived MMPs. MMP-8, also referred to as collagenase-2 is the most prevalent MMP in diseased periodontal tissue and GCF and has a key role in degrading the triple helical structures of types I, II, and III collagens found in alveolar bone (Birkedal-Hansen, 1993). In addition to release from PMNs, mesenchymal cells, such as human gingival and periodontal ligament fibroblasts and chondrocytes produce MMP-8 (Chubinskaya et al., 1996). Opinion is divided in terms of the potential usefulness of MMP-8 as a biomarker of disease. Elevated MMP-8 levels have been observed in GCF (Ingman et al., 1996) and saliva (Ingman et al., 1996; Rai et al., 2008) of periodontitis patients. However no significant differences in GCF MMP-8 levels between healthy and periodontitis subjects were observed using a checkerboard immunoblotting technique (Teles et al., 2009b). Further studies are required to evaluate MMP-8 either alone or in conjunction with other biomarkers to predict the risk of future disease occurrence and to monitor treatment interventions. Of the other family members, MMP-2, MMP-3 and MMP-9, have also been determined in saliva of periodontitis patients (Ingman et al., 1996). However given the abundance of MMP-8, less emphasis has been placed on the other MMP family members as potential biomarkers of disease.

Aspartate Aminotransferase

Aspartate aminotransferase (AST) belongs to a family of intracellular enzymes that are released from damaged cells of periodontal tissues into GCF and saliva. However, the high prevalence of AST-positive sites detected during gingival inflammation diminishes this biomarker's ability to discriminate between progressive and stable, but inflamed, sites (Oringer et al., 2001).

ECM Molecules and Metabolites

Proteoglycans are integral components of the noncollagenous ECM with diverse roles in regulating activity of growth factors and proteases, collagen formation, ECM assembly, cell adhesion and growth.

Decorin is one of the major proteoglycans secreted by human periodontal fibroblasts*in vitro* (Larjava et al., 1992), and is expressed abundantly in the periodontal connective tissues *in vivo.* Breakdown of collagen-rich connective tissue matrix in periodontal disease parallels a decrease in the level of decorin in the inflamed connective tissue (Oksala et al., 1997). Decreased decorin levels may arise as a result of increased decorin degradation caused by

inflammation (Waddington et al., 1998) or by down-regulated decorin expression by fibroblasts. Degradation products originating from the active destruction of the alveolar bone could prove to be effective biomarkers for active destruction of alveolar bone if robust assays could be developed for their determination.

Further work on ECM degradation products has been possible as a result of increasingly sophisticated 'omic' technologies. Metabolomics, the systematic study of the unique chemical fingerprints that specific cellular processes leave behind, is a powerful tool for biomarker discovery in bodily fluids including GCF. It represents an unbiased approach to studying the cellular metabolites of the host pathogen interaction in periodontal disease. Metabolomic profiling of GCF has yielded over 200 metabolites with altered levels associated with periodontal disease status (Barnes et al., 2009). Some of these metabolites, including representatives of the purine degradation pathway (a major biochemical source for reactive oxygen species production), were significantly accelerated at disease sites and responded to a triclosan-containing dentifrice in a 6-week clinical study (Barnes et al., 2010). Four of the markers, inosine, lysine, putrescine, and xanthine, could be very sensitive indicators for periodontal status and should be investigated further as putative biomarkers.

Biomarkers of Bone Turnover

Alveolar bone loss is a key discriminator between gingivitis and periodontitis and remains an important area for biomarker discovery.

Alkaline Phosphatise (ALP)

ALP is released from PMNs during the inflammatory response (McCulloch, 1994), from osteoblasts during bone formation (Christenson, 1997) and from periodontal ligament fibroblastsduring periodontal regeneration (Groenveld et al., 1997). ALP activity in GCF is thought to reflect periodontal recurrent inflammation or healing phases in chronic periodontitis patients (Chapple et al., 1999). GCF ALP activity has been proposed as a marker of successful treatment in chronic periodontitis since this activity was shown to decrease as early as 15 days following scaling and root planning and (Perinetti et al., 2008).

Osteocalcin

Osteocalcin is a small, noncollagenous, highly conserved, secreted protein that is associated with the mineralized matrix of bone. Elevated serum levels of osteocalcin have been found during periods of rapid bone turnover, such as during fracture repair. Studies have shown that GCF osteocalcin levels alone are unable to discriminate between active and inactive sites (Nakashima et al., 1996).

Osteopontin

Osteopontin is a single-chain polypeptide with a molecular weight of approximately 32,600 daltons. It is produced by both osteoblasts and osteoclasts, and as a result has a role in both bone maturation/mineralization as well as bone resorption. Osteopontin has been shown to correlate with clinical measures of disease (Kido et al., 2001). Further research should be directed to determining osteopontin's potential as a biomarker of disease progression.

Osteonectin

Osteonectin is a 32,000 dalton bone-specific protein that binds selectively to both hydroxyapatite and collagen, and has been implicated in the early phases of tissue mineralization. In a cross-sectional study of osteonectin in GCF, osteonectin levels appeared to be a sensitive marker for detection of periodontal disease status (Bowers et al., 1989).

Pyridinoline Cross-Linked Carboxyterminal Telopeptide of Type I Collagen (ICTP)

Following the synthesis of procollagen and its release into the ECM, collagen fibrils undergo a series of posttranslational modifications some of which result in cross-link formation between the telopeptide regions of type I collagen. Pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP) is derived from the carboxyterminal telopeptide regions of type I collagen cross-linked via pyridinoline or deoxypyridinoline (Risteli et al., 1993). These ICTP cross links are essential for providing mechanical stability to the ECM and more importantly are specific to bone and cartilage. ICTP levels in GCF have been shown to be significantly elevated in periodontitiscompared to gingivitis or periodontally healthy subjects (Palys et al., 1998). ICTP levels correlated with clinical parameters and putative periodontal pathogens, demonstrating significant reductions after periodontal therapy (Palys et al., 1998). Given the specificity of ICTP measurement for bone resorptionfurther work should be undertaken to develop ICTP measurements as putative biomarkers for periodontal disease.

Combinations of Biomarkers

The use of multiple biomarkers has been employed for many years to improve the diagnostic or prognostic potential of individual markers. Combination of osteocalcin, collagenase, PGE_2, alpha-2 macroglobulin, elastase and alkaline phosphatase was shown to increase diagnostic sensitivity and specificity values to 80 and 91%, respectively (Offenbacher et al., 1986). With advancements in novel technologies the ease of multiple biomarker measurements has improved. Multiple combinations of salivary biomarkers (MMP-8, MMP-9 and osteoprotegerin) combined with red-complex anaerobic periodontal pathogens (such as *Porphyromonas gingivalis* or *Treponema denticola*) provided highly accurate predictions of periodontal disease category (Ramseier et al., 2009).

Conclusion

At present periodontitis is diagnosed visually and through periodontal probing and radiographs at which stage the diagnosis is retrospective, after irreversible destruction has occurred. A validated biomarker for periodontial disease could be used to diagnose disease, monitor response to therapy, identify sites at risk of progression and assist in determining personalised therapeutic intervention and recall.Whether such biomarkers should be measured in GCF or saliva depends very much on the biochemical characteristics of the putative biomarker as does the requirement for simple detection or level above a certain concentration

threshold for positivity. The potential for multiple biomarker analysis to improve sensitivity and specificity adds another layer of complexity. The revolution in 'omic' technologies holds much promise for biomarker discovery and should lead to new avenues for future research. However challenges still remain for development of affordable chair side assays and given that such biomarkers are likely to predict the probability of disease rather than certainty of disease it is expected that clinical judgement and experience will still be relied upon, at least in the medium term.

References

Anon. (2003). Academy Report. Position Paper. Diagnosis of periodontal diseases. *Journal of Periodontology* 74, 1237-1247.

Bachrach, G., Muster, Z., Raz, I., Chaushu, G., Stabholz, A., Nussbaum, G., Gutner, M., Chaushu, S. (2008). Assessing the levels of immunoglobulins in the saliva of diabetic individuals with periodontitis using checkerboard immunodetecion. *Title Oral Diseases* 14, 51-59.

Barnes, V.M., Teles, R., Trivedi, H.M., Devizio, W., Xu, T., Mitchell, M.W., Milburn, M.V., Guo, L. (2009). Acceleration of purine degradation by periodontal diseases. *Journal of Dental Research* 88, 851-855.

Barnes, V.M., Teles, R., Trivedi, H.M., Devizio, W., Xu, T, Lee. D.P, Mitchell, M.W., Wulff, J.E., Milburn, M.V., Guo, L. (2010) Assessment of the effects of dentifrice on periodontal disease biomarkers in gingival crevicular fluid. *Journal of Periodontology.* Epub ahead of print(doi:10.1902/jop.2010.100070)

Bickel, M., Cimasoni, G., Andersen, E. (1985). Flow and albumin content of early (pre-inflammatory) gingival crevicular fluid from human subjects. *Archives of Oral Biology* 30, 599-602.

Birkedal-Hansen, H. (1993). Role of matrix metalloproteinases in human periodontal diseases. *Journal of Periodontology* 64, 474–484.

Bowers, M.R., Fisher, L.W., Termine J.D., Somerman M.J. (1989). Connective tissue-associated proteins in crevicular fluid: potential markers for periodontal diseases. *Journal of Periodontology* 60, 448–451.

Chapple, I.L., Garner, I., Saxby, M.S., Moscrop, H., Matthews, J.B. (1999). Prediction and diagnosis of attachment loss by enhanced chemiluminescent assay of crevicular fluid alkaline phosphatase levels. *Journal of Clinical Periodontology* 26, 190-198.

Christenson, R.H. (1997). Biochemical markers of bone metabolism: An overview. *Clinical Biochemistry* 30, 573-593.

Chubinskaya, S., Huch, K., Mikecz, K., Cs-Szabo, G., Hasty, K.A., Kuettner, K.E., Cole, A.A. (1996). Chondrocyte matrix metalloproteinase-8: up-regulation of neutrophil collagenase by interleukin-1 beta in human cartilage from knee and ankle joints. Laboratory Investigation 74, 232–240.

Curtis, M.A., Gillett, I.R., Griffiths, G.S., Maiden, M.F.J., Sterne, J.A.C., Wilson, D.T., Wilton, J.M.A., Johnson N.W. (1989). Detection of high-risk groups and individuals for periodontal diseases. Laboratory markers from analysis of gingival crevicular fluid. *Journal of Clinical Periodontology* 16, 1-11.

Curtis, M.A., Griffiths, G.S., Price, S.J., Coulthurst, S.K., Johnson, N.W. (1988). The total protein concentration of gingival crevicular fluid. Variation with sampling time and gingival inflammation. *Journal of Clinical Periodontology* 15, 628-632.

Ebersole, .J.L, Holt, S.C. Periodontal Diseases. Markers of disease susceptibility and activity. In: Johnson, N.W. (Editor). Risk Markers for Oral Diseases Volume 3. Cambridge: Cambridge University Press; 1991; 203-227.

Eley, B.M., Cox, S.W. (2006). Cathepsin B/L-, elastase-, tryptase-, trypsin- and dipeptidyl peptidase IV-like activities in gingival crevicular fluid: Correlation with clinical parameters in untreated chronic periodontitis patients. *Journal of Periodontal Research*27, 62-69.

Fitzsimmons, T.R., Sanders, A.E., Bartold, P.M., Slade, G.D. (2010). Local and systemic biomarkersin gingival crevicular fluid increase odds of periodontitis. *Journal of Clinical Periodontology* 37,30–36.

Frodge, B.D., Ebersole, J.L., Kryscio, R.J., Thomas, M.V., Miller, C.S. (2008). Bone remodelling biomarkers of periodontal disease in saliva. *Journal of Periodontology* 7, 1913-1919.

Genco, R.J. Pathogenesis and host responses in periodontal disease. In: Genco R, Goldman H, Cohen D. (Editors). Contemporary Periodontics. Saint Louis: CV Mosby Company; 1990; 184-193.

Groeneveld, M.C., Van den Bos, T., Everts, V., Beertsen, W. (1996). Cell-bound and extracellular matrix-associated alkaline phosphatase activity in rat periodontal ligament. *Journal of Periodontal Research* 31, 73-79.

Heasman, P., Lauffart, B.L., Presfiaw, P.M. (1998). Crevicular fluid prostaglandin E2 levels in periodontitis-resistant and periodontitis-susceptible adults. *Journal of Clinical Periodontology* 25, 1003-1007.

Ingman, T., Tervahartiala ,T., Ding, Y., Tschesche, H., Haerian, A., Kinane, D.F., Konttinen, Y.T., Sorsa, T. (1996) Matrix metalloproteinases and their inhibitors in gingival crevicular fluid and saliva of peridontitis patients. *J. Clin. Perio.* 23, 1127-1132.

Jeffcoat, M.K., Jeffcoat, R.L., Jens, S.C., Captain, K. (1986). A new periodontal probe with automated cemento-enamel junction detection. *Journal of Clinical Periodontology*13, 276-280.

Kennett, C.N., Cos, S.W., Eley, B.M. (1997). Investigations into the cellular contribution to host tissue proteases and inhibitors in gingival crevicular fluid. *Journal of Clinical Periodontology* 24, 424–431.

Kido, J., Nakamura, T., Asahara, Y., Sawa, T., Kohri, K., Nagata T. (2001). Osteopontin in gingival crevicular fluid. *Journal of Periodontal Research* 36, 328–333.

Kido, J., Nakamura, T., Kido, R., Ohishi, K., Yamauchi, N., Kataoka M., Nagata, T. (1999). Calprotectin in gingival crevicular fluid correlates with clinical and biochemical markers of periodontal disease. *Journal of Clinical Periodontology* 26,653–657.

Kornman, K.S., Page, R.C., Tonetti, M.S. (1997). The host response to the microbial challenge in periodontitis: assembling the players. *Periodontology* 2000 14, 33–53.

Kunimatsu, K., Mine, N., Muraoka, Y., Kato, I., Hase, T., Aoki, Y., Yamamoto, K. (1995). Identification and possible function of cathepsin G in gingival crevicular fluid from chronic adult periodontitis patients and from experimental gingivitis subjects. *Journal of Periodontal Research* 30, 51-57.

Kunimatsu, K., Yamamoto, K., Ichimaru, E., Kato, Y, Kato I. (1990). Cathepsins B, H and L activities in gingival crevicular fluid from chronic adult periodontitis patients and experimental gingivitis subjects. *Journal of Periodontal Research* 25, 69–73.

Lamster, I.B. (1997) Evaluation of components of gingival crevicular fluid as diagnostic tests. *Annals of Periodontology* 2, 123-137.

Lang, N.P., Alder, R., Joss, A., Nyman, S. (1990). Absence of bleeding on probing. An indicator of periodontal stability. *Journal of Clinical Periodontology* 17, 714-721.

Larjava, H., Häkkinen, L., Rahemtulla, F. (1992). A biochemical analysis of human periodontal tissue proteoglycans. *Biochemical Journal* 284, 267–274.

Linden, G.J., McKinnell, J., Shaw, C., Lundy, F.T. (1997). Substance P and neurokinin A in gingival crevicular fluid in periodontal health and disease. *Journal of Clinical Periodontology*24, 799-803.

Linden, G.J., Mullally, B.M., Burden, D.J., Lamey, P.-J., Shaw, C., Ardill, J., Lundy, F.T. (2002). Changes in vasoactive intestinal peptide in gingival crevicular fluid in response to periodontal treatment. *Journal of Clinical Periodontology*29, 484-489.

Loos, B.G., Tjoa, S. (2005). Host-derived diagnostic markers for periodontitis: do they exist in gingival crevice fluid? *Periodontology* 2000 39. 53–72.

Lundy, F.T., Chalk, R., Lamey, P.-J., Shaw, C., Linden, G.J. (2000). Identification of MRP-8 (Calgranulin A) as a major responsive protein in chronic periodontitis. *Journal of Pathology*, 192, 540-544.

Lundy, F.T., Chalk, R., Lamey, P.-J., Shaw, C., Linden, G.J. (2001). Quantitative analysis of MRP-8 in gingival crevicular fluid in periodontal health and disease. *Journal of Clinical Periodontology*, 28, 1172-1177.

Lundy, F.T., Linden, G.J. (2004). Neuropeptides and neurogenic mechanisms in oral and periodontal inflammation. *Critical Reviews in Oral Biology and Medicine* 15, 82-98.

Lundy, F.T., El Karim, I., Linden, G.J. (2009). Neuropeptide Y (NPY) and NPY Y1 receptor in periodontal health and disease. *Archives of Oral Biology* 54, 258-262.

Lundy, F.T., Shaw, C., McKinnell, J., Lamey, P.-J., Linden, G.J. (1999). Calcitonin gene-related peptide in gingival crevicular fluid in periodontal health and disease. *Journal of Clinical Periodontology*26, 212-216.

Luthman, J., Johansson, O., Ahlström, U., Kvint, S. (1988). Immunohistochemical studies of the neurochemical markers, CGRP, enkephalin, galanin, gamma-MSH, NPY, PHI, proctolin, PTH, somatostatin, SP, VIP, tyrosine-hydroxylase and neurofilament in nerves and cells of the human attached gingiva. *Archives of Oral Biology* 33,149-158.

McCauley, L.K., Nohutcu, R.M. (2002). Mediators of periodontal osseous destruction and remodelling: principles and implications for diagnosis and therapy. *Journal of Periodontology* 73, 1377– 91.

McCulloch, C.A. (1994). Host enzymes in gingival crevicular fluid as diagnostic indicators of periodontitis. *Journal of Clinical Periodontology* 21, 497-506.

Mogi, M., Otogoto, J. (2007). Expression of cathepsin-K in gingival crevicular fluid of patients with periodontitis. *Archives of Oral Biology*52, 894-898.

Nakashima, K., Giannopoulou, C. , Andersen, E., Roehrich, N., Brochut, P., Dubrez, B., Cimasoni, G. (1996). A longitudinal study of various crevicular fluid components as markers of periodontal disease activity. *Journal of Clinical Periodontology* 233, 832–838.

Nisapakultorn, K., Ross, K.F.,Herzberg M.C. (2001). Calprotectin expression *in vitro* by oral epithelial cells confers resistance to infection by *Porphyromonasgingivalis*. *Infection and Immunity* 69, 4242–4247.

Offenbacher, S., Odle B.M., Van Dyke T.E. (1986). The use of crevicular fluid prostaglandin E2 levels as a predictor of periodontal attachment loss. *Journal of Periodontal Research* 21, 101–112.

Oksala, O., Haapasalmi, K., Häkkinen, L., Uitto, V.J., Larjava, H. (1997). Expression of heparan sulphate and small dermatan/chondroitin sulphate proteoglycans in chronically inflamed human periodontium. *Journal of Dental Research* 76, 1250–1259.

Oringer, R.J., Howell, T.H., Nevins, M.L., Reasner, D.S., Davis, G.H., Sekler, J., Fiorellini, J.P. (2001). Relationship between crevicular aspartate aminotransferase levels and periodontal disease progression. *Journal of Periodontology* 72,17-24.

Palys, M.D., Haffajee, A.D., Socransky, S., Giannobile, W.S. (1998). Relationship between C-telopeptide pyridinoline cross-links (ICTP) and putative periodontal pathogens in periodontitis. *Journal of Clinical Periodontology* 25, 865-871.

Perinetti, G., Paolantonio, M., Femminella, B., Serra, E., Spoto, G. (2008). Gingival Crevicular Fluid Alkaline Phosphatase Activity Reflects Periodontal Healing/Recurrent Inflammation Phases in Chronic Periodontitis Patients. *Journal of Periodontolgy* 79, 1200-1207.

Rai, B., Kharb, S., Jain, R., Anand, S.C. (2008). Biomarkers of periodontitis in oral fluids. *Journal of Oral Science* 50, 53-56.

Ramseier, C.A., Kinney, J.S., Herr, A.E., Braun, T., Sugai, J.V., Shelburne, C.A., Rayburn, L.A., Tran, H.M., Singh, A.K., Giannobile, W.V. (2009). Identification of pathogen and host-response markers correlated with periodontal disease. 80, 436-446.

Risteli, J., Elomaa, I., Niemi, S., Novamo, A., Risteli, L. (1993). Radioimmunoassay for the pyridinoline cross-linked carboxyterminal telopeptide of type I collagen: a new serum marker of bone collagen degradation. *Clinical Chemistry*39,635–640.

Teles, R.P., Likhari, V., Socransky, S.S., Haffajee, A.D. (2009a). Salivary cytokine levels in subjects with chronic periodontitis and in periodontally healthy individuals: a cross-sectional study. *Journal of Periodontal Research* 44, 411–417.

Teles, R.P., Sakellari, D., Konstantinidis, A., Socransky, S.S., Haffajee A.D. (2009b). Application of the Checkerboard Immunoblotting Technique to the Quantification of Host Biomarkers in Gingival Crevicular Fluid. *Journal of Periodontology* 80, 447-456.

Tsai, C.C., Hong, Y.C., Chen, C.C., Wu, Y.M. (1998). Measurement of prostaglandin E2 and leukotriene B4 in the gingival crevicular fluid. *Journal of Dentistry* 26, 97 –103.

Waddington, R.J., Embery, G., Smith, A.J. (1998). Immunochemical detection of the proteoglycans decorin and biglycan in human gingival crevicular fluid from sites of advanced periodontitis. *Archives of Oral Biology* 43, 287–295.

Wilton, J.M.A, Griffiths, G.S., Curtis, M.A., Maiden M.F.J., Gillett, I.R., Wilson D.T., Sterne, J.A.C., Johnson, N.W. (1988). Detection of high-risk groups and individuals for periodontal diseases. *Journal of Clinical Periodontology* 15, 339-346.

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Chapter V

Inflammatory Mediators and Oxidative Stress in Periodontal Disease

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Abstract

Periodontal disease represents today the main cause of teeth loss after the third decade of life. About 60% of dental extractions are due to etiopathogenetic periodontal factors. After 35 years, the frequency of marginal periodontal disease varies from 80% to 100% of world population, depending on statistical method used and the demographic areas considered, showing a similar frequency in both sexes, slightly higher in female.

Two important and interrelated factors are involved in its physiopathological progression: 1) the activation of immune system and the release of inflammatory mediators, such as IL-1β, IL-6 and TNF-α, which could overflow into the blood system and induce a systemic inflammatory response; 2) the production of oxygen radicals and their related metabolites.

A recent focus of the dental research is the individuation of biomarkers, which can be easily used as diagnostic tools. Among them, metalloproteinases (MMPs) and heat

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shock proteins (HSPs) could provide potential biomarkers, which could be useful for evaluating both the periodontitis development and the incidence of the related cardiovascular diseases. Recent studies, in fact, have shown a direct correlation between periodontal and cardiovascular diseases: in particular, both diseases have systemic and local causes, and the constant bacterial contamination of oral cavity could be linked not only to periodontopathy but also to the development of cardiovascular diseases.

To date, the periodontal disease therapy available is based on the individuation and the elimination of the causing factors. Nevertheless, new innovative surgical and pharmacological therapies could be developed.

The aim of this work is to review the literature data focusing on the role of inflammatory mediators and oxidative stress in periodontal disease and related factors.

Introduction

Periodontal disease is now the leading cause of tooth loss after the third decade of life: about 60% of dental avulsion, in fact, can be traced back to periodontitis. Over 60% of the world population in the most industrialized countries is afflicted with a form of moderate periodontitis: only about 20% of these cases present serious injury that would irreversibly impair the dental health. After the age of 35, the marginal incidenceof periodontal disease varies between the 80% and 100% of the world population, with a slightly higher frequency in women then men.

Periodontitis is characterized by gingival inflammation and often results in periodontal pocket formation with loss of the supporting alveolar bone and connective tissue around the teeth (Raja et al., 2009). Regarding the biological mechanism leading this pathology, it reflects the interplay between a pathogenic bacterial biofilm present on the root surface/periodontal pocket, host-derived inflammatory cells and molecules from periodontal tissue(Kinane and Lappin, 2002; Page, 1999). Two important and interrelated factors are involved in its progression: the physiopathological activation of immune system and the production of oxygen radicals and their related metabolites (Sorry, 2009).

This paper aims to highlight, by analysis and description of previous studies, the role of inflammatory mediators and oxidative stress in periodontal disease.

1. Periodontal Diseases and Dental Practice

From the clinical point of view, the periodontal disease is usually classified according to the age of the patient, the histological finding and some other criteria.

It is already perfectly known that the periodontal disease is a multifactor disease, which can have also a genetic involvement; so, the patient genetic predisposition could be an important aspect for the clinician in order to better calibrate the treatment options (Pihlstrom, 2001).

Following the concepts and guidelines of the Royal College of Surgeons about “Standards in Dentistry”, periodontal disease should be clinically classified in three levels, taking into consideration different important aspects, such as a) description b) assessment c) non-surgical treatment, d) surgical treatment, e) patient compliance and f) continuing care.

- *GRADE A:* a) any periodontal procedure where there is potential for a loss of attachment or an increase in inflammation of the gingival tissue; no regard paid to individual treatment of the areas of the mouth requiring attention; neglect of oral hygiene; b) a causal visual inspection of the periodontal tissues and any radiographs without reference to any established objective criteria; no attempt to record any measures of disease state of activity; no periodontal indices taken; risk factors not assessed; c) failure to apply a periodontal screening system and to respond effectively to its indications; some hard and soft deposits that could have been removed have been left behind on root surfaces following a scaling and root surface debridement (cleaning); d) failure to apply a periodontal screening system and to respond effectively to its indications; sub-gingival or periodontal surgical procedure performed where adjacent supra-gingival plaque control is not consistently excellent; the surgical procedure has not been effective in correcting the condition it was intended to resolve; e) failure by the patient to understand the nature the problem; a wish to have the dentist or hygienist solve the problem without further effort on the patient's part; f) the dentist does not screen all periodontal sites at recall visits; any data that are recorded fail to allow for visit by visit comparison; no attempt is made to provide a recall programme for periodontal care.
- *GRADE B:* a) generally healthy gingival tissues with tooth surfaces free from hard and soft deposits; any bleeding on probing from pocket depths greater than 4 mm is being monitored with a view to surgery if applicable; fewer than six sites exhibit gingival bleeding; b) routine screening has been performed using the basic periodontal examination (BPE) followed by systematic inspection of all periodontal sites and appropriate radiographs of the periodontium, where appropriate; careful recording of selected indices showing the status of the periodontium; c) regular uniform removal of hard and soft deposits by patient and operator; progressive elimination of all pockets and plaque retention factors by means of careful scaling, root surface debridement and other mechanical means, in conjunction with good plaque control; d) periodontal surgery has been effective in correcting the condition it was carried out to resolve; correct regular uniform removal of hard and soft deposits by patient and operator now possible; the patient may still find areas difficult to clean; the final gingival contour is not ideal; there may be some aesthetic and dentinal sensitivity problems post-operatively; e) the patient is willing to attend, as required, for periodontal screening to attempt to understand the nature of the problem and to undertake a plaque control programme as directed; the patient's hygiene programme tends to be disrupted by outside events, both social and work-related; f) the dentist has instituted a simple periodontal screening programme examining all periodontal sites in all patients; there is a continuing commitment to the programme by all members of the practice; the monitoring systems of response when "trigger" points are reached; the practice has a regular recall system based on the NICE (National Institute Clinical Excellence) guidelines.
- *GRADE C:* a) healthy gingival tissues with tooth surfaces free from hard and soft deposits; no pocket probing depth than 4 mm and no bleeding on probing from any residual pockets or from the gingival tissue; b) a systematic inspection of all periodontal sites supported by necessary radiographs; indices recorded in a visual

form which can be used for explanations to the patient; appropriate indices selected following an initial screening index such as with the BPE; c) elimination of all bleeding points and bleeding pockets by meticulous removal of hard and soft deposits by the dentist or hygienist, and meticulous plaque control by the patient; d) periodontal surgery has been effective in correcting the condition it was intended to resolve; regular uniform removal of hard and soft deposits by patient and operator now possible; the patient can clean all areas easily, and the final gingival contour is ideal; any post-operative aesthetic and dentinal sensitivity problems have been treated effectively; e) the patient is eager to attend as required for frequent monitoring; the patient has a commitment to understand the nature of the problem in considerable detail; the patient regularly practises plaque control to the highest levels attainable, with sufficient skill to avoid tooth or gingival damage; the patient usually arranges both social life and work to prevent a substantial interruption of their recall programme; f) the practice tailors all recall programmes to the needs of individual patients and the NICE guidelines and provides comprehensive monitoring; a system of support of patient's plaque control procedures exists, using professional staff; the practice tolerates only the earliest signs of disease activity before initiating a suitable response.

The clinical considerations and/or treatments are often based on the personal skill and knowledge of the clinicians; consequently, they result more theoreticalin relation with the real patient attitude and interest. Oral hygiene and its maintenance are a typical example that can clarify this concept: in fact, if the patient does not care about the problem and its consequences, the best treatment performed by the best clinical could undergo to a big failure. These are the reasons why we strongly believe that a classification based on these principles could be more reliable and applicable to the everyday evidence based dentistry. So, having some on inflammatory mediatorbased index and check the degree of the periodontal disease should be more important and helpful.

2. Dental Hygiene and Periodontal Disease

Epidemiological studies have demonstrated a significant association between the severity of periodontal disease, the amount of dental plaque and the level of oral hygiene, with a cause/ effect relationship between the formation and accumulation of dental plaque and the development of periodontitis (Cabanilla and Molinari, 2009; Timmerman and van der Weijden, 2006).

The diagnosis and the classification of periodontal disease as moderate, severe and refractory may be made by the dentist thanks to a full documentation of clinical parameters and intraoral periapical radiographs (Corbet et al. 2009; Herzog and Paarmann, 1997).Moreover, during the local inspection, it is essential to evaluate also the following parameters: the level of oral hygiene by detecting the presence of plaque and tartar with specific "plaque index"; the presence of local predisposing factors such as retained restorations/incongruous prosthetics, anomalous shape and position of teeth, occlusal trauma; local signsof soft tissues inflammation (i.e. swelling, soreness/tenderness, bleeding tendency),

using the bleeding index or bleeding on probing (BOP); the presence of periodontal lesions (i.e periodontal attachment loss, alveolar bone recession).

The collaboration between the dentist and the dental hygienist is essential in the treatment of periodontal disease to ensure the healing of periodontal tissues and to monitor the progression of healing processes, since recurrent disease could develop during the phase treatment (Shumaker et al., 2009; Stabholzet al., 1998).

To date, the periodontal disease therapy available is based on various approaches, including simple oral hygiene practices, professional mechanical debridement, antimicrobial therapy(topic or systemic)and periodontal surgery.

As above reported the periodontitis is promoved by microbial biofilm which could lead to an inflammatory status and cause an imbalance in the red-ox status, resulting in oxidative stress-induced damage (Soory, 2009). With normal oral health and dental care,only small numbers of mostly facultative bacterial species gainaccess to the bloodstream. However, with poor oral hygiene, thenumbers of bacteria colonizing the teeth, could increase (Loesche, 1997) and thus possibly introducemore bacteria into tissue and the bloodstream, leading to an increasein the prevalence and magnitude ofbacteremia (Li et al., 2000). Consequently, good oral hygiene is essential both in preventing and treating periodontal disease (Renz and Newton, 2009) and so it is related to cardiovascular diseases (Scannapieco et al., 2010; Lockhart et al., 2009).

Assistance provided by dental hygienists includes removing deposits of plaqueor calcified bacterial material (calculus) that mayprevent effective self-care, as well as dealing with anyassociated secondary aetiological factors, such as smoking or dietary control (Cercek et al., 2007; Rosen et al., 1999; Magnusson et al., 1984).

3. Epidemiology and Etiopathogenesis of Periodontal Diseases: Risk Factors and Oxidative Stress

Human periodontal disease is an inflammatory disorder that is the result of etiological multiple factors, involving systemic as well as local conditions.

The main etiopathogenic agents in periodontal disease are anaerobic bacteria Gram-negative, which are present in dental plaque (Bascones Martinez and Figuero Ruiz, 2005; Marsh, 2003; Socransky and Haffajee, 2002). These bacteria play an important role, participating in the formation of the periodontal pocket, connective tissue and periodontal ligament destruction and alveolar bone resorptiondirectly by the production of toxic products and indirectly byactivating host defense systems, i.e. inflammation, which affect gingival tissue(Kinney et al., 2007; Page and Kornman, 1997).

One irritating agent, a lipopolysaccharide (LPS), is a major constituent of the outer bacterial membrane and a critical determinant in pathology development (Offenbacher, 1996). Once periodontitis has been established, an inflammatory infiltrate is formed, consisting of different kinds of cells, such as macrophages and lymphocytes, which will produce different cytokines, including interleukin (IL)-1β, tumor necrosis factor (TNF)-α and other biological mediators (Raja et al., 2009; Wei et al., 2004; Alexander et al., 1994).

One more important factor involved in the development of periodontal disease is the imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses, which lead to oxidative stress (Wei et al., 2004; Battino et al., 1999).

The molecular mechanism of ROS-caused tissue damage includes peroxidation of membrane lipids, modification of protein (enzyme) and stimulation of pro-inflammatory cytokine release (Chapple, 1997).

Most studies focused on the changes that the oxidative stress induces in the immune system (Soory, 2009; Canakci et al., 2005;Wei et al., 2004;Biondi and Zannino, 1997). Several lines of evidence implicate polymorphonuclearcells (PMNs) as the primary mediators ofthe host response against periodontopathic bacteria, by the production of a range of antimicrobial factors, including ROS, duringthe phagocytosis of periodontopathic bacteria (Canakci et al., 2009; Jenkinson and Dymock, 1999).

Immune defense against antigens depends by a complex immuno-neuro-endocrine network in which cortisol from the adrenal cortex and cytokines (e.g. IL-1β, IL-6) play an important role as interactive mediators (Kemeny and Grünewald, 1999). From a "microbial endocrinology" point of view, the potentially pathogenic microorganisms would be able to recognize hormones as stimuli to their growth and proliferation, thus initiating their pathogenicity (Lyte, 1993). So, the stress could determine a synergic effect by decreasing the immune system host response and increasingthe bacterial pathogenicity in the oral cavity. Consequently, an increased concentration of catecholamine, which occurs as a reaction of the body to stress, is effective in promoting oxidative tissue damage as well as growth and virulence of a wide range of pathogens (Lyte, 1997).

In relation with the main role of oxidative stress in periodontal disease, recently, several studies have shown that antioxidant compounds, such as melatonin, suppress both inflammation and oxidative stress, suggesting that they could act as protective molecules against fighting periodontal infection (Zdarilova et al., 2010; Gomez-Moreno et al., 2007).

However, the following risk factors have to be also considered: age, race, gender, general dysgnathia, acquired habits (smoking, alcoholism, etc.), disorders (diabetes mellitus, heart failure, dysendocrinism, hematological diseases and autoimmune diseases, etc.), iatrogenic factors (incongruous prosthetic plates, drugs, etc.) and socio-economic hardship.

In the last decade the genetic factor has also emerged.There is a growing evidence that polymorphisms in the IL-1, IL-6, IL-10, vitamin D receptor, and CD14 genes may be associated with chronic periodontitis in adulthood (Laine et al., 2010; Kornman and Duff, 2001). Moreover, the genetic factor could be explaining the association between periodontal disease and cardiovascular disease, since it influences both pathologies. At present, one candidate that influences inflammation, IL-1 gene polymorphisms, has been associated with periodontal disease and cardiovascular disease (Stein et al., 2009).

4. Correlation between Periodontal and Cardiovascular Diseases: Role of Oxidative Stress and Inflammatory Response

Recent studies point to the attention on a possible correlation between chronic and severe periodontitis and cardiovascular diseases, such as diabetes mellitus, hyperlipidemia,

atherosclerosis and ischemic heart disease (Ekuni et al., 2009; Stein et al., 2009; Chun et al., 2005;Cutler and Iacopino, 2003; Li et al., 2000). Since in industrialized countries the incidence of cardiovascular disease is still the leading cause of death, the confirmation that chronic periodontitis represents a real contributory cause for cardiovascular diseases could has a significant importance for public health.

In this respect there are interesting studies that support a direct clinical correlation between chronic periodontal lesion and high risk of acute myocardial infarction (Dorn et al., 2010; Stein et al., 2009; DeStefano et al., 1993; Mattila et al., 1989).

Levels of risk markers for cardiovascular diseases, such as glucose, C-reactive protein and IL-18, have been reported to be elevated in patients with periodontitis (Buhlin et al., 2009a; Buhlin et al., 2009b; Loos, 2005). Moreover, the release of host-derived inflammatory mediators, such as cytokines (IL-1β, IL-6, TNF-α) and bacterial products from the chronically inflamed periodontal tissue into the blood stream might lead to a systemic inflammatory response, such as acute-phase proteins, and immune effectors including systemic antibodies to periodontal bacteria (Albandar et al., 2001;Genco, 1996), providing a link between periodontal and cardiovascular diseases.

Three pathways linking oral infections to systemic response effects have been proposed: metastatic spread of infection as a result of transient bacteraemia, metastatic injury from the effects of circulating oral microbial toxins and metastatic inflammation caused by an injury induced by oral microorganisms (Losche et al. 2000; Meyer and Fives-Taylor, 1998).

Considering the correlation between periodontal and cardiovascular diseases, the diagnosis of chronic periodontitis and the identification of the risk factors represent a challenge for clinical evaluations. Clinical and radiographic assessment of periodontal disease remains the basis for patient evaluation; nevertheless, many studies have focused on the identification of markers in saliva, crevicular fluid and blood (Goncalves et al., 2010; Greabu et al., 2006; Wei et al., 2004; Kaufman and Lamster, 2000).

Furthermore, there is increasing of evidence about the link between periodontal and inflammatory diseases driven by pro-oxidant profile. Recent studies in experimental periodontitis showed that the excessive ROS production in periodontal diseases diffuses into the blood stream (Tomofuji et al., 2009) and that the circulating oxidative stress can impair the salivary gland function and have a systemic effects on other organs (Ekuni et al., 2010). Oxidative stress, in fact, makes a significant contribution to a variety of human diseases, such as heart diseases, stroke, diabetes (Boesing et al., 2009; McCord, 2000), causing DNA and protein damage, lipid peroxidation, stimulating the proinflammatory cytokines and activating nuclear factor kB (NFkB) (Wei et al., 2004; Chapple 1997; Renard et al., 1997), which regulates various genes that are important in inflammatory response (Lee an Burckart, 1998).

According to these data, periodontal disease may be a useful marker of a susceptible immune system and can be directly related with the progression of systemic diseases due to inflammatory and oxidative stress loading.

5. Local Biomarkers and Histological Alteration in Periodontal Diseases

Periodontitis is an inflammatory disease of the tissue surrounding and supporting the teeth that results in a progressive destruction of gingival tissue, periodontal ligament and alveolar bone (Dutzan et al., 2009; Philstrom et al., 2005).

A recent focus of the dental research is the individuation of biomarkers, which can be easily used as diagnostic tools. Among them, number of publications underlined the implication of matrix metalloproteinase (MMPs) (Giannobile, 2008). MMPs play an important role in the degradation of various extracellular molecules, including collagen, elastin, proteoglycan and laminins and in tissue remodeling associated with various physiological and pathological processes, such as morphogenesis, tooth development, angiogenesis, wound healing, arthritis, chronic heart failure, chronic obstructive pulmonary disease, chronic inflammation and cancer metastasis (Dorman et al., 2010; Ra and Parks, 2007).

In healthy gingival tissue, the connective tissue is a well-organized fibrillar network composed of different collagenous components, each having a specific localization and functional role (Borsani et al., 2005). Types I and III collagens produced by periodontal ligament and gingival fibroblasts are the predominant extracellular matrix components of periodontium (Borsani et al., 2005; Birkedal-Hansen, 1993).

Several studies have reported that the assessment of MMP levels in oral fluids, such as gingival crevicularfluid, peri-implant sulcular fluid, mouth-rinses and saliva,during periodontalinflammation reflects the degree ofthe pathological periodontal collagen catabolism and can be utilized to develop new non-invasive, chair-side, point-of-care diagnostics for periodontitis and dental peri-implantitis (Biyikoğlu et al., 2009; Sorsa et al., 2006; Collin et al., 2000). MMP-1, MMP-3, MMP-8 and MMP-13 are the principal neutralproteinases capable of degrading native collagen fibers in the extracellular space and the majorcollagenase species detected in inflamed humanperiodontium (Sorsa et al., 2006; Tervahartiala et al., 2000;Freije et al., 1994; Birkedal-Hansen, 1993). Their expression and activity in non-inflamed periodontium is low but is drastically enhanced in relation with dental plaque and infection-induced periodontal inflammation. Recent studies suggest that MMPs are not only implicated in the cascade of events involving bacterial virulence but they can also exert anti-inflammatory effects in defense of the host by processing anti-inflammatory cytokines and chemokines, as well as by regulating apoptotic and immune responses(Sorsa et al., 2006).

These results point the attention on the use of MMPs as promising candidates for predicting, diagnosis and assessing of the periodontal diseases (Herr et al., 2007).

Whereas some studies have focused on the alteration of extracellular matrix, relative few studies have investigated the involvement of stress protein in periodontal diseases, considering the main role of oxidative stress in the etiopathogenesis of this disease. Stress proteins are a super-family of proteins, known as heat-shock proteins (HSPs) that are highly conserved from prokaryotes to mammals. They function mainly as molecular chaperones and control the correct folding of new synthesized proteins (Borsani et al., 2007).

These proteins are classified according to their molecular weight and structural characteristics. Among them, HSP25 and HSP27 are small proteins that, generally, provide an

important index of cancer evolution, fibrosis, oxidative damage and tooth development (Onishi et al., 2002; Rogalla et al., 1999); HSP32 has an antioxidant effect by catalyze the oxidative degradation of heme to biliverdin and then bilirubin (Abraham, 2003); HSP60 is present in the inner mitochondrial membranes as a mitochondrial chaperone (Soltys and Gupta, 1999) and it has been increasingly recognized as an important molecule in infectious and autoimmune diseases (Ueki et al., 2002); HSP72 is an inducible isoform enhanced by specific stress (Suzuki et al., 1998).

HSPs are produced by a wide variety ofbacteria and human cells under a variety of stressful orharsh conditions such as high temperature, infection,inflammation, and mechanical stress(Benjamin and McMillan, 1998).

Many HSPsof oral micro-organisms, particularly periodontopathogens, havebeen identified.The cytotoxicity of some bacterial HSPs may contributeto tissue destruction, whereas the presence of common epitopesin host proteins and microbial HSPs may lead to autoimmune responses (Goulhen et al., 2003).

Since these proteins areimmunodominant antigens in many human pathogens, studies haverecently focused on the potential contributions of HSPs to oraldiseases.

Moreover, sinceseveral pathologicalfunctions have been associated with these proteinstheir presence could be used as a prognostic index in several diseases; in particular, serological differencesin subjects with periodontitis have been reported (Mattila, 2003; Buhlin et al., 2009a), providing new insight into the epidemiological association between periodontitis and cardiovascular diseases.

References

Abraham, NG. Therapeutic applications of human heme oxygenase gene transfer and gene therapy. *Curr. Pharm Des*, 2003 9, 2513-2524.

Albandar, JM; DeNardin, AM; Adesanya, MR; Diehl, SR; Winn, DM. Associations between serum antibody levels to periodontal pathogens and early-onset periodontitis. *J. Periodontol*, 2001 72, 1463-1469.

Alexander, MB; Damoulis, PD. The role of cytokines in the pathogenesis of periodontal diasease. *Curr. Opin. Periodontol*, 1994 39, 53.

Bascones Martinez, A; Figuero Ruiz, E. Periodontal diseases as bacterial infection. *Av. Periodon. Implantol*, 2005 17, 111-118.

Battino, M; Bullon, P; Wilson, M; Newman, II. Oxidative injury and inflammatory periodontal diseases, the challenge of antioxidants to free radicals and reactive oxygen species. *Crit. Rev. Oral. Biol. Med*, 1999 10, 458-476.

Benjamin, IJ; McMillan, DR. Stress (heat shock) proteins: molecular chaperones in cardiovascular biology and disease. *Circ. Res*, 1998 83, 117-132.

Biondi, M; Zannino, LG. Psychological stress, neuroimmunomodulation, and susceptibility to infectious diseases in animals and man: a review. *Psychother Psychosom*, 1997 66, 3-26.

Birkedal-Hansen, H. Role of cytokines and inflammatory mediators in tissue destruction. *J. Periodontal Res*, 1993 28, 500-510.

Biyikoğlu, B; Buduneli, N; Kardeşler, L; Aksu, K; Pitkala, M; Sorsa, T. Gingival crevicular fluid MMP-8 and -13 and TIMP-1 levels in patients with rheumatoid arthritis and inflammatory periodontal disease. *J. Periodontol*, 2009 80, 1307-1314.

Boesing, F; Patiño, JS; da Silva, VR; Moreira, EA. The interface between obesity and periodontitis with emphasis on oxidative stress and inflammatory response. *Obes. Rev*, 2009 10, 290-297.

Borsani, E; Salgarello, S; Mensi, M; Boninsegna, R; Stacchiotti, A; Rezzani, R; Sapelli, P; Bianchi, R; Rodella, LF. Histochemical and immunohistochemical evaluation of gingival collagen and metalloproteinases in peri-implantitis. *Acta Histochem*, 2005 107, 231-240.

Borsani, E; Salgarello, S; Stacchiotti, A; Mensi, M; Boninsegna, R; Ricci, F; Zanotti, L; Rezzani, R; Sapelli, P; Bianchi, R; Rodella, LF. Altered immunolocalization of heat-shock proteins in human peri-implant gingiva. *Acta Histochem*, 2007 109, 221-227.

Buhlin, K; Hultin, M; Norderyd, O; Persson, L; Pockley, AG; Rabe, P; Klinge, B; Gustafsson, A. Risk factors for atherosclerosis in cases with severe periodontitis. *J. Clin. Periodontol*, 2009a 36, 541-549.

Buhlin, K; Hultin, M; Norderyd, O; Persson, L; Pockley, AG; Pussinen, PJ; Rabe, P; Klinge, B; Gustafsson; A. Periodontal treatment influences risk markers for atherosclerosis in patients with severe periodontitis. *Atherosclerosis*, 2009b 206, 518-522.

Cabanilla, L; Molinari, G. Clinical considerations in the management of inflammatory periodontal diseases in children and adolescents. *J. Dent. Child (Chic)*, 2009 76, 101-108.

Canakçi, CF; Ciçek, Y; Canakçi, V. Reactive oxygen species and human inflammatory periodontal diseases. *Biochemistry (Mosc)*, 2005 70, 619-28.

Canakçi, CF; Canakçi, V; Tatar, A; Eltas, A; Sezer, U; Ciçek, Y; Oztas, S. Increased salivary level of 8-hydroxydeoxyguanosine is a marker of premature oxidative mitochondrial DNA damage in gingival tissue of patients with periodontitis. *Arch Immunol. Ther. Exp. (Warsz)*, 2009 57, 205-211.

Cercek, JF; Kiger, RD; Garret, S; Egelberg, J. Relative effects of plaque control and instrumental on the clinical parameters of human periodontal disease. *J. Clin. Periodontol*, 2007 10, 46–56.

Chapple, ILC. Reactive oxygen species and antioxidants in inflammatory diseases. *J. Clin. Periodontol*, 1997 24, 287-296.

Chun, YH; Chun, KR; Olguin, D; Wang, HL. Biological foundation for periodontitis as a potential risk factor for atherosclerosis. *J. Periodontal Res*, 2005 40, 87-95.

Collin, HL; Sorsa, T; Meurman, JH; Niskanen, L; Salo, T; Rönkä, H; Konttinen, YT; Koivisto, AM; Uusitupa, M. Salivary matrix metalloproteinase (MMP-8) levels and gelatinase (MMP-9) activities in patients with type 2 diabetes mellitus. *J. Periodontal Res*, 2000 35, 259-265.

Corbet, EF; Ho, DK; Lai, SM. Radiographs in periodontal disease diagnosis and management. *Aust. Dent. J*, 2009 54, S27-S43.

Cutler, CW; Iacopino, AM. Periodontal disease: links with serum lipid/triglyceride levels? Review and new data. *J. Int. Acad. Periodontol*, 2003 5, 47-51.

DeStefano, F; Anda, RF; Kahn, HS; Williamson, DF; Russell, CM. Dental disease and risk of coronary heart disease and mortality. *BMJ*, 1993 306, 688-691.

Dormán, G; Cseh, S; Hajdú, I; Barna, L; Kónya, D; Kupai, K; Kovács, L; Ferdinandy, P. Matrix metalloproteinase inhibitors: a critical appraisal of design principles and proposed therapeutic utility. *Drugs*, 2010 70,949-964.

Dorn, JM; Genco, RJ; Grossi, SG; Falkner, KL; Hovey, KM; Iacoviello, L; Trevisan, M. Periodontal disease and recurrent cardiovascular events in survivors of myocardial infarction (MI): the Western New York Acute MI Study. *J. Periodontol*, 2010 81, 502-511.

Dutzan, N; Vernal, R; Hernandez, M; Dezerega, A; Rivera, O; Silva, N; Aguillon, JC; Puente, J; Pozo, P; Gamonal, J. Levels of interferon-gamma and transcription factor T-bet in progressive periodontal lesions in patients with chronic periodontitis. *J. Periodontol*, 2009 80, 290-296.

Ekuni, D; Tomofuji, T; Sanbe, T; Irie, K; Azuma, T; Maruyama, T; Tamaki, N; Murakami, J; Kokeguchi, S; Yamamoto, T. Periodontitis-induced lipid peroxidation in rat descending aorta is involved in the initiation of atherosclerosis. *J. Periodontal Res*, 2009 44, 434-442.

Ekuni, D; Endo, Y; Irie, K; Azuma, T; Tamaki, N; Tomofuji, T; Morita, M. Imbalance of oxidative/anti-oxidative status induced by periodontitis is involved in apoptosis of rat submandibular glands. *Arch Oral Biol*, 2010 55, 170-176.

Freije, JM; Díez-Itza, I; Balbín, M; Sánchez, LM; Blasco, R; Tolivia, J; López-Otín, C. Molecular cloning and expression of collagenase-3, a novel human matrix metalloproteinase produced by breast carcinomas. *J. Biol. Chem*, 1994 269, 16766-16773.

Genco, RJ. Current view of risk factors for periodontal diseases. *J. Periodontol*, 1996 67, 1041-1049.

Giannobile. WV. Host-response therapeutics for periodontal diseases. *J. Periodontol*, 2008 79, 1592-1600.

Gómez-Moreno, G; Cutando-Soriano, A; Arana, C; Galindo, P; Bolaños, J; Acuña-Castroviejo, D; Wang, HL. Melatonin expression in periodontal disease. *J. Periodontal Res*, 2007 42, 536-540.

Gonçalves Lda, R; Soares, MR; Nogueira, FC; Garcia, C; Camisasca, DR; Domont, G; Feitosa, AC; Pereira Dde, A; Zingali, RB; Alves, G. Comparative proteomic analysis of whole saliva from chronic periodontitis patients. *J. Proteomics*, 2010 73, 1334-1341.

Goulhen, F; Grenier, D; Mayrand, D. Oral microbial heat-shock proteins and their potential contributions to infections. *Crit. Rev. Oral Biol. Med*, 2003 14, 399-412.

Greabu, M; Purice, M; Totan, A; Spînu, T; Totan, C. Salivary cortisol-marker of stress response to different dental treatment. *Rom. J. Intern. Med*, 2006 44, 49-59.

Herzog, A; Paarmann, C. Enhancing accurate assessment of periodontal disease by improving radiographic interpretation. *Probe*, 1997 31, 130-135.

Herr, AE; Hatch, AV; Throckmorton, DJ; Tran, HM; Brennan, JS; Giannobile, WV; Singh, AK. Microfluidic immunoassays as rapid saliva-based clinical diagnostics. *Proc. Natl. Acad. Sci. USA*, 2007 104, 5268-5273.

Jenkinson, HF; Dymock, D. The microbiology of periodontal disease. *Dent Update*, 1999 26, 191-197.

Kaufman, E; Lamster, IB. Analysis of saliva for periodontal diagnosis--a review. *J. Clin. Periodontol*, 2000 27, 453-465.

Kemeny, ME; Gruenewald, TL. Psychoneuroimmunology update. Semin *Gastrointest Dis*, 1999 10, 20-29.

Kinane, DF; Lappin, DF. Immune processes in periodontal diseases: a review. *Ann. Periodontol*, 2002 7, 62-71.

Kinney, JS; Ramseier, CA; Giannobile, WV. Oral fluid-based biomarkers of alveolar bone loss in periodontitis. *Ann. N. Y. Acad. Sci*, 2007 108, 230-251.

Kornman, KS; Duff, GW. Candidate genes as potential links between periodontal and cardiovascular diseases. *Ann. Periodontol*, 2001 6, 48-57.

Laine, ML; Loos, BG; Crielaard, W. Gene polymorphisms in chronic periodontitis. *Int. J. Dent*, 2010 2010, 324719.

Lee, JI; Burckart, GJ. Nuclear factor kappa B: important transcription factor and therapeutic target. *J. Clin. Pharmacol*, 1998 38, 981-993.

Li, X; Kolltveit, KM; Tronstad, L; Olsen, I. Systemic diseases caused by oral infection. *Clin. Microbiol. Rev*, 2000 13, 547-558.

Lockhart, PB; Brennan, MT; Thornhill, M; Michalowicz, BS; Noll, J; Bahrani-Mougeot, FK; Sasser, HC. Poor oral hygiene as a risk factor for infective endocarditis-related bacteremia. *J. Am. Dent. Assoc*, 2009 140, 1238-1244.

Loesche, WJ. Association of the oral flora with important medical diseases. *Curr. Opin. Periodontol*, 1997 4, 21-28.

Loos, BG. Systemic markers of inflammation in periodontitis. *J. Periodontol*, 2005 76, 2106-2115.

Lösche, W; Karapetow, F; Pohl, A; Pohl, C; Kocher, T. Plasma lipid and blood glucose levels in patients with destructive periodontal disease. *J. Clin. Periodontol*, 2000 27, 537-541.

Lyte, M. The role of microbial endocrinology in infectious disease. *J. Endocrinol*, 1993 137, 343-345.

Lyte, M. Induction of gram-negative bacterial growth by neurochemical containing banana (Musa x paradisiaca) extracts. *FEMS Microbiol. Lett*, 1997 154, 245-250.

Magnusson, I; Lindhe, J; Yoneyama, T; Liljenberg, B. Recolonization of a subgingival microbiota following scaling in deep pockets. *J. Clin. Periodontol*, 1984 11, 193–207.

Marsh, PD. Plaque as a biofilm: pharmacological principles of drug delivery and action in the sub- and supragingival environment. *Oral Dis*, 2003 9, 16-22.

Mattila, K; Rasi, V; Nieminen, M; Valtonen, V; Kesäniemi, A; Syrjälä, S; Jungell, P; Huttunen, JK. von Willebrand factor antigen and dental infections. *Thromb Res*, 1989 56, 325-329.

Mattila K. Does periodontitis cause heart disease? *Eur. Heart J*, 2003 24, 2079-2080.

McCord, JM. The evolution of free radicals and oxidative stress. *Am. J. Med*, 2000 108, 652-659.

Meyer, DH; Fives-Taylor, PM. Oral pathogens: from dental plaque to cardiac disease. *Curr. Opin. Microbiol*, 1998 1, 88-95.

Offenbacher, S. Periodontal diseases: pathogenesis. *Ann. Periodontol*, 1996 1, 821-878.

Onishi, T; Tsubone, H; Ooshima, T; Sobue, S; El-Sharaby, A; Wakisaka, S. Immunohistochemical localization of heat shock protein 25 (HSP 25) during root formation of the rat molar. *Anat. Rec*, 2002 267, 321-329.

Page, RC; Kornman, KS. The pathogenesis of human periodontitis: an introduction. *Periodontol. 2000*, 1997 14, 9-11.

Page RC. Millestones in periodontal research and the remaining critical issue. *J. Periodontol. Res*, 1999 34, 331-339.

Pihlstrom, BL. Periodontal risk assessment, diagnosis and treatment planning. *Periodontol. 2000*, 2001 25, 37-58.

Pihlstrom, BL; Michalowicz, BS; Johnson, NW. Periodontal diseases. *Lancet*, 2005 366, 1809-1820.

Ra, HJ; Parks, WC. Control of matrix metalloproteinase catalytic activity. *Matrix Biol*, 2007 26, 587-596.

Raja, S; Byakod, G; Pudakalkatti, P. Growth factor in periodontal regeneration. *Int. J. Den. Hygiene*, 2009 7, 82-89.

Renard, P; Zachary, MD; Bougelet, C; Mirault, ME; Haegeman, G; Remacle, J; Raes, M. Effects of antioxidant enzyme modulations on interleukin-1-induced nuclear factor kappa B activation. *Biochem. Pharmacol*, 1997 53, 149-160.

Renz, AN; Newton, JT. Changing the behavior of patients with periodontitis. *Periodontol. 2000*, 2009 51, 252-268.

Rogalla, T; Ehrnsperger, M; Preville, X; Kotlyarov, A; Lutsch, G; Ducasse, C; Paul, C; Wieske, M; Arrigo, AP; Buchner, J; Gaestel, M. Regulation of Hsp27 oligomerization, chaperone function, and protective activity against oxidative stress/tumor necrosis factor alpha by phosphorylation. *J. Biol. Chem*, 1999 274, 18947-18956.

Rosen, RW; Olavi, G; Badersten, A; Ronstrom, A; Soderholm, G; Egelberg, J. Effect of different frequencies of preventive maintenance treatment on periodontal conditions. 5-year observations in general dentistry patients. *J. Clin. Periodontol*, 1999 26, 225–233.

Scannapieco, FA; Dasanayake, AP; Chhun, N. "Does periodontal therapy reduce the risk for systemic diseases?". *Dent. Clin. North Am*, 2010 54, 163-181.

Shumaker, ND; Metcalf, BT; Toscano, NT; Holtzclaw, DJ. Periodontal and periimplant maintenance: a critical factor in long-term treatment success. *Compend Contin. Educ. Dent*, 2009 30, 388-390.

Socransky, SS; Haffajee, AD. Dental biofilms: difficult therapeutic targets. *Periodontol. 2000*, 2002 28, 12-55.

Soltys, BJ; Gupta, RS. Mitochondrial-matrix proteins at unexpected locations: Are they exported? *Trends Biochem. Sci*, 1999 24, 174-177.

Sorry, M. Redox status in periodontal and systemic inflammatory conditions including associated neoplasias: antioxidant as adjunctive therapy? *Infect Disord Drug Targets*, 2009 9, 415-427.

Sorsa, T; Tjäderhane, L; Konttinen, YT; Lauhio, A; Salo, T; Lee, HM; Golub, LM; Brown, DL; Mäntylä, P. Matrix metalloproteinases: contribution to pathogenesis, diagnosis and treatment of periodontal inflammation. *Ann. Med*, 2006 38, 306-321.

Stabholz, A; Mann, J; Berkey, D. Periodontal health and the role of the dental hygienist. *Int. Dent. J*, 1998 48, 50-55.

Stein, JM; Smeets, R; Reichert, S; Chrobot, J; Fickl, S; Stanzel, S; Kuch, B. The role of the composite interleukin-1 genotype in the association between periodontitis and acute myocardial infarction. *J. Periodontol*, 2009 80, 1095-1102.

Suzuki, K; Kodama, S; Watanabe, M. Effect of low-dose preirradiation on induction of the HSP70B-LacZ fusion gene in human cells treated with heat shock. *Radiat. Res*, 1998 149, 195-201.

Tervahartiala, T; Pirilä, E; Ceponis, A; Maisi, P; Salo, T; Tuter, G; Kallio, P; Törnwall, J; Srinivas, R; Konttinen, YT; Sorsa, T. The in vivo expression of the collagenolytic matrix metalloproteinases (MMP-2, -8, -13, and -14) and matrilysin (MMP-7) in adult and localized juvenile periodontitis. *J. Dent. Res*, 2000 79, 1969-1977.

Timmerman, MF; van der Weijden, GA. Risk factors for periodontitis. *Int. J. Dent. Hyg*, 2006 4, 2-7.

Tomofuji, T; Ekuni, D; Irie, K; Azuma, T; Endo, Y; Tamaki, N; Sanbe, T; Murakami, J; Yamamoto, T; Morita, M. Preventive effects of a cocoa-enriched diet on gingival oxidative stress in experimental periodontitis. *J. Periodontol*, 2009 80, 1799-1808.

Ueki, K; Tabeta, K; Yoshie, H; Yamazaki, K. Self-heat shock protein 60 induces tumour necrosis factor-alpha in monocyte-derived macrophage: possible role in chronic inflammatory periodontal disease. *Clin. Exp. Immunol*, 2002 127, 72-77.

Wey, PF; Ho, KY; Ho, YP; Wu, YM; Yang, YH; Tsai, CC. The investigation of glutathione peroxidase, lactoferrin, myeloperoxidase and interleukin-1β in gingival crevicular fluid: implications for oxidative stress in human periodontal diseases.*J. Periodont. Res*, 2004 39, 287-293.

Zdarilova, A; Rajnochova Svobodova, A; Chytilova, K; Simanek, V; Ulrikova, J. Polyphenolic fraction of *Lonicera cerulea* L. fruits reduces oxidative stress and inflammatory markers induced by lipopolysaccharide in gingival fibroblasts. *Food and Chem. Toxicol*, 2010 48, 1555-1561.

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Chapter VI

Tobacco: A Risk Factor for Periodontal Disease

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Abstract

Periodontal disease results from complex interactions between infectious agents and host factors. The disease expression can be modified by environmental, acquired, and genetic risk factors. Tobacco usage, especially smoking, is considered a major modifiable risk factor for periodontal disease. In addition to periodontal disease, tobacco usage is also a risk factor for oral cancer and its recurrence, dental cariesand congenital defects in children from mothers who smoke while pregnant. In periodontal disease, smokers have deeper probing depths, more gingival recession, more alveolar loss and more furcation involvement than non-smokers. They also show less favorable responses to various kinds of periodontal treatments including non-surgical, surgical, regenerative procedures and dental implants. It is clear from epidemiology studies that tobacco usage is correlated with periodontal disease. This chapter reviews the evidence for the association between periodontal disease and tobacco, and describes what is currently known about how tobacco and its components affect the periodontal tissues that result in tissue damage.

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Introduction

The association between smoking and periodontal disease has been well documented clinically and the concept that tobacco use is detrimental to periodontal health is not new [1]. The relationship between smoking and the various forms of periodontal disease has been established in numerous studies [2-5]. Currently, there is a large body of scientific evidence that both smokers and smokeless tobacco users have an increased risk, incidence and severity of periodontal disease as made evident by increased gingival recession, tooth loss, and periodontal destruction.

Smokers are almost three to four times more at risk of developing severe periodontitis than non-smokers[6]. One of the largest epidemiological studies [7] was reported by the National Health and Nutrition Examination Survey (NHANES III) on smoking and periodontal disease. Almost 42% of periodontitis cases among smokers in the NHANES III study were attributable to current cigarette smoking and 10.9% to former smoking [7]. After adjustments for age, race, socioeconomic and educational levels, current smokers were four times more likely to develop periodontitis than non-smokers [7].

The oral cavity is the first part of the human body to be exposed to the mainstream smoke in active smokers, thus smoking could have deleterious effects on the teeth and oral mucosa. The oral cavity can be exposed to up to 1000 μg of nicotine after smoking one cigarette [8]. The plasma nicotine levels were reported to be in the range of 15–73 ng/ml after the use of tobacco products, while the nicotine levels were determined to be as high as 1560 μg/ml in saliva samples from smokeless tobacco users [9].

This chapter reviews the evidence for the association between periodontal disease and tobacco, and describes what is currently known about how tobacco and its components affects the periodontal tissues that results in tissue damage.

Effects of Smoking on Periodontal Tissue

Many studies have shown that tobacco smoking has a direct influence on the prevalence and severity of periodontal disease[10-13]. Smokers tend to have more calculus[14], more plaque[15] and poorer standards of oral hygiene than nonsmokers[16, 17]. Smokers also tend to have less gingival bleeding than nonsmokers and less signs of inflammation[18]. The decreased inflammation could be due to vasocontstriction of the gingival vessels[19] and may also be attributed to the heavy keratinization of the gingiva in smokers. Holmes noted a decrease in the gingival crevicular fluid (GCF) flow in smokers when compared with nonsmokers [20]. A study by Shirodaria [21] on the GCF of smokers reported a reduction in interleukin-1α (IL-1α) compared to nonsmokers. IL-1 is known to be an important mediator of the inflammatory and the immune response.

Smokers have deeper probing depths [3], more deep pockets and more attachment loss [22], as well as more gingival recession[22]. Smokers also have more alveolar bone loss [23], more teeth with furcation involvement [24, 25] and suffer more tooth loss than non-smokers[26, 27]. The patterns of attachment loss have indicated more loss in the maxillary palatal areas, thus suggesting the possibility of a local effect too [28].

Numerous studies have indicated that the severity of periodontal disease significantly correlates with the number of cigarettes smoked per day and the duration (years) of smoking[29, 30]. The severity of bone loss has also been positively correlated to smoking [31]. It was noted by Martinez-Canut et al. that smoking one cigarette per day, up to 10, and up to 20 increased clinical attachment loss by 0.5%, 5% and 10%, respectively [32].

Acute necrotizing gingivitis (ANUG) is a severe form of periodontal disease identified over 50 years ago [10]. ANUG is a rapidly progressive, painful, necrotizing microbial lesion of the gingival tissues typically affecting young heavy smoking adults. Kowolik et al. [33] found that 98 out of 100 individuals with ANUG were smokers, while Stammers [34] examined 1017 cases of ANUG and reported that almost all of them were smokers. These studies clearly demonstrate a strong and definite association between ANUG and smoking. The exact relationship and the underlying mechanisms are not clear. However, some local and systemic factors have been suggested such as poor oral hygiene and stress [35, 36].

Smokeless tobacco users also display loss of gingival tissues and destruction of the alveolar bone[37]. The underlying mechanism appears to be excessive collagen breakdown due to increased levels of the matrix metalloproteinases (MMPs). Moreover, smokeless tobacco has been strongly linked to oral leukoplakias and carcinomas [38]. These are commonly found in areas of the mouth where the tobacco product was placed.

Effects of Smoking on the Subgingival Microflora

Numerous studies found no significant differences between smokers and nonsmokers for the presence of *Actinobacillus actinomycemcomitans*[39-41], *Prevotella intermedia* [39, 40, 42], *Fusibacterium nucleatum* [39, 41], *Eikenella corrodens* [41], *Treponema denticola* [42], *and Porphyromonas gingivalis (P. gingivalis)* [39-42]. In contrast, Zambon et al. [31] investigated the relationship between periodontal pathogens and cigarette consumption in 1,426 subjects and reported that smokers had an increased risk of 2.3 and 3 times that of non-smokers or former smokers to harbor *Bacteroides forsythus* and *Actinobacillus actinomycetemcomitans*, respectively. Moreover, they reported an increased risk for smokers to have subgingival infections with *P. gingivalis*, although it was not statistically significant.

In addition to subgingival microflora, other studies have reported a dose-dependent relationship between the number of cigarettes smoked and bacterial levels in saliva[43]. *Streptococcus mutans* tend to increase its growth rate in smokers [44]. Recently, it has been reported that the gene expression of several important adhesion factors and virulence activities of *Streptococcus mutans* are increased with cigarette smoke condensate (CSC) and nicotine[45, 46].

Effects of Smoking on Periodontal Therapy

In regards to non-surgical therapy, smokers have less successful treatments than do nonsmokers[30, 47]. It has been shown that the differences between smokers and non-smoker become more prominent and significant in probing depths ≥5 mm, where smokers demonstrated less improvement in clinical attachment levels following scaling and root planning [48]. Palmer et al. showed that even with the use of adjunctive systemic or locally applied antibiotics (i.e., metronidazole) that smokers had poorer treatment responses to non-surgical therapies [49].

Smokers responded less favorably to flap debridement surgery in terms of pocket depth reduction and attachment level gains [50]. It has also been reported that smoking reduced attachment level gains after papilla preservation flaps even when heavy smokers were excluded from the study [51]. For root coverage procedures, smokers had significantly less root coverage when guided tissue regeneration procedures or subepithelial connective tissue graft were used [52, 53]. Similar findings were reported in the combined use of allografts with guided tissue regeneration for the treatment of intrabony defects[54] and molar furcation defects [55].

The survival of osseo-integrated implants was significantly influenced by the smoking status of patients [56]. Smoking may also make the patient more susceptible to peri-implantitis [57]. A 6-year study demonstrated that a significantly greater percentage of implants failures occurred in smokers (11.28%) than in nonsmokers (4.76%) [58]. Complications after implant placement were reported to be higher in smokers, especially in implants with high cover screws and were also related to smoking duration [59, 60].

Although smokers will also benefit from treatment, but to a lesser degree, treatment failures tend to predominate among smokers [61]. Accordingly, all periodontal patients and particularly potential surgical candidates should be actively discouraged from smoking.

Effects of Nicotine on Cells of the Periodontium

Theilig et al. reported that nicotine can be rapidly taken up by human keratinocytes[62]. It penetrates the epithelial barrier of the oral mucosa where it can be retained and metabolized. Cultured keratinocytes increased IL-1α concentrations when exposed to nicotine [63]. In addition, El Ahmer et al. showed enhanced bacterial binding to epithelial cells in smokers [64]. It was postulated that some of the components in the smoke was capable of altering specific properties of the surface of the epithelial cells [65]. Moreover, pure nicotine was able to decrease the synthesis of lactoferrin, secretory components and lyzozyme of human epithelial cells [66]. Giannopoulou et al. showed that nicotine-treated epithelial cells inhibited the proliferation, total protein, collagen production and non-collagen protein production of gingival fibroblasts[65]. It was not determined which factor(s) released by the epithelial cells was/were responsible for the changes in the fibroblast cells.

Human gingival fibroblasts (HGFs) are the major connective tissue cells in the gingiva [67]. They are responsible for the synthesis and degradation of the extracellular matrix through the production of the extracellular matrix-degrading enzymes such as the MMPs. The MMPs are a group of zinc-dependent endopeptidases that include the collagenases, gelatinases, stromelysins, membrane-associated MMPs and others [68]. Zhou and Windsor [69] reported that nicotine enhanced the collagen-degrading ability of HGFs in a dose dependent manner. The collagen degradation was clearly visible when the HGFs were treated with 150 and 250 µg/ml of nicotine. When HGFs were treated with 150 or 250 µg/ml of nicotine, proMMP-2 underwent increased activation as revealed by the zymography of the conditioned media from HGFs [69]. Using Western blots, MMP-14, MMP-2 and TIMP-2 were detected in both the conditioned media and membrane extracts from the treated and untreated HGFs. A lower-molecular-weight fragment of MMP-14, as well as a partially activated form of MMP-2, were more prevalent in the membrane extracts of the nicotine-treated HGFs. There was also an increase in the level of tissue inhibitor of metalloproteinases (TIMP-2) in the membrane extracts and a decrease in its level in the conditioned media from the HGFs. The HGF-mediated collagen degradation was mediated by the MMPs since it was completely inhibited by an MMP inhibitor, GM6001 [69]. When HGFs were treated with both nicotine and *P. gingivalis*, an additive effect in regard to collagen cleavage was observed [69]. HGFs also increased the expression levels of multiple cytokines/growth factors when exposed to nicotine or *P. gingivalis* lipopolysaccharides [70]. Expression levels of IL-10, IL-6, IL-7, IL-8 and Monocyte Chemotactic Protein-1 were increased when the HGFs were treated with nicotine or *P. gingivalis* lipopolysaccarides [70].

Nicotine has been shown to have an inhibitory effect on fibroblast attachment to root surfaces and thus may interfere with the healing process after periodontal therapy[71]. Tanur et al. [72] evaluated the effects of nicotine on the strength of attachment of HGFs to glass and non-diseased human root surfaces. They reported disruption of the normal orientation of the cells on dentin and glass surfaces, as well as increased cell vacuolization when exposed to different concentrations of nicotine [72]. Nicotine also had adverse effects on periodontal ligament cell proliferation, attachment and chemotaxis[73, 74].

Fang et al. [75] demonstrated that nicotine inhibited myofibroblast differentiation in HGFs *in vitro*. Myofibroblasts are the major cells that lead to wound contraction to bring wound margins together. This study supports the hypothesis that delayed wound healing in smokers may be due to decreased wound contraction by myofibroblasts[75].

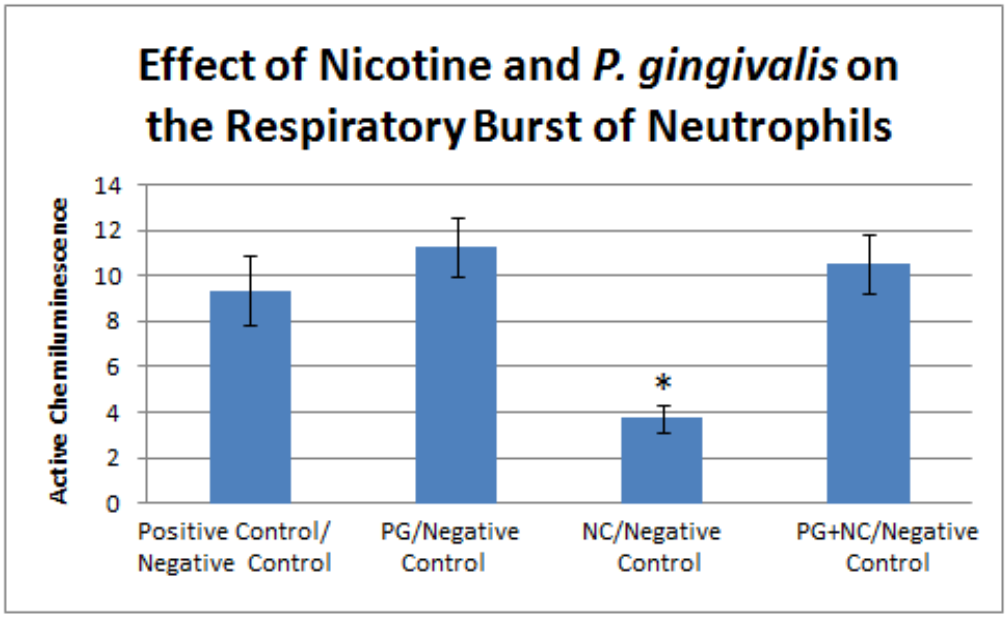
Effects of Nicotine on Cells of the Immune System

Neutrophils play an important role in the innate immune response against bacteria, fungi and other pathogens in the oral cavity. Neutrophil-mediated bacterial killing involves oxygen-dependent and oxygen–independent processes. The oxygen dependent process is represented by the respiratory burst by which neutrophils kill the phagocytosed bacterial cells through the generation of multiple reactive oxygen and reactive nitrogen species[76]. Several studies have suggested that cigarette smoke components may inhibit the respiratory burst of the neutrophils [77, 78]. A compromised respiratory burst can reduce the ability of neutrophils to

destroy the bacteria. However, lipopolysaccharides from periodontal pathogens stimulate the respiratory burst of neutrophils [79]. Using chemiluminescence assays, the *in vitro* stimulation of neutrophils with 10% *P. gingivalis* and 80 µg/ml of nicotine together masked the effects of nicotine and increased the respiratory burst at a same level as *P. gingivalis* alone with a *p-value* of 0.9889 (Figure 1). Neutrophils stimulated with nicotine alone had a much lower chemiluminescence than *P. gingivalis* or *P. gingivalis* and nicotine with a *p-value* of < 0.0001. The positive control used in this study was neutrophils stimulated with N-formyl-methionyl-leucyl-phenylalanine, while the negative control was a blank that contained no neutrophils (Figure 1).

Tobacco smoke exposure has been reported to increase the number of neutrophils in the systemic circulation[80], but it seems not to affect the number of neutrophils entering the gingival sulcus and the oral cavity. Functional receptors for nicotine, cotinine and aryl hydrocarbons are expressed on neutrophils [81, 82]. The numbers of these receptors are increased in smokers and decline on cessation [83]. Seow et al. examined the effects of nicotine on neutrophil function and showed a dose dependent suppression of both chemotaxis and phagocytosis[84].

Tobacco smoking has been reported to contribute to a significant increase in the circulating numbers of neutrophil elastase and the MMPs[85]. Therefore, it is possible that tobacco leads to the progression of periodontal disease partly by the induction of proteases released from the neutrophils. Mariggio et al. [86] exposed neutrophils to nicotine concentrations ranging from 0.01 to 0.3% and reported apoptosis of the neutrophils.



* Denotes statistically significant value ($p<0.05$).

Figure 1. Effects of nicotine and P. gingivalis on the respiratory burst of neutrophils. The positive control (32962 ± 4907), P. gingivalis (37806 ± 3915), and P. gingivalis + nicotine (34298 ± 3726) groups had significantly higher active chemiluminescence than did the nicotine group (13041 ± 1893), all with p values <0.0001. The P. gingivalis and P. gingivalis + nicotine groups were not significantly different from each other (p=0.99).

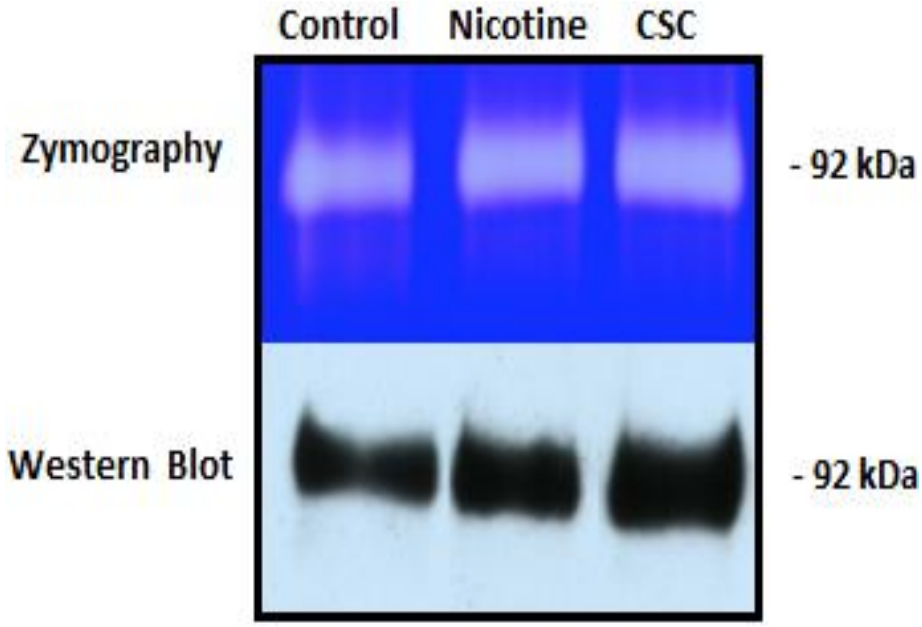


Figure 2. Effects of nicotine and cigarette smoke condensate on neutrophil's release of MMP-9. Zymography and Western Blot analyses for MMP-9 in the conditioned media from neutrophils treated with either 100 µg/ml of nicotine or 50 µg/ml of CSC for 1 hour.

The authors hypothesized that the nicotine could dysregulate apoptosis of the first cells recruited in the host defense strategy of the periodontium, and then making them ineffective functionally [86].Treatment of neutrophils with either 100 µg/ml of nicotine or 50 µg/ml of CSC increased the release of MMP-9 after 1 hour of incubation (Figure 2)**.** The nicotine and CSC-treated neutrophils increased the release of MMP-9 by 116% and 145% respectively after 1 hour of treatment when compared to the control as determined by Western Blot.

The effects of tobacco smoking on T cell function and proliferation is controversial. Several studies have shown significant reductions in T cell proliferation, while others have reported no significant differences in the proliferative rate of T cells in smokers and non-smokers[87]. In regards to B cells, they seemed to be similar in numbers in smokers and non-smokers, but their function was impaired in smokers [88]. Animals exposed to smoke showed a reduction in antigen-induced proliferation [88]. Upon cessation of smoking, B cell function returned to normal [89].

Effects of Nicotine on the Vascular Tissue

Nicotine has been directly related to the effects that smoking has on the cardiovascular system, including the development of arterial disease. A study by Park et al. demonstrated that endothelial cells, which lines vascular blood vessels, when exposed to nicotine cause various changes in their cellular behavior [90]. Human umbilical vein endothelial cells (HUVECs) showed a significant decrease in cell proliferation when the cells were treated with 400 and 800 µg/ml of nicotine (Figure 3).

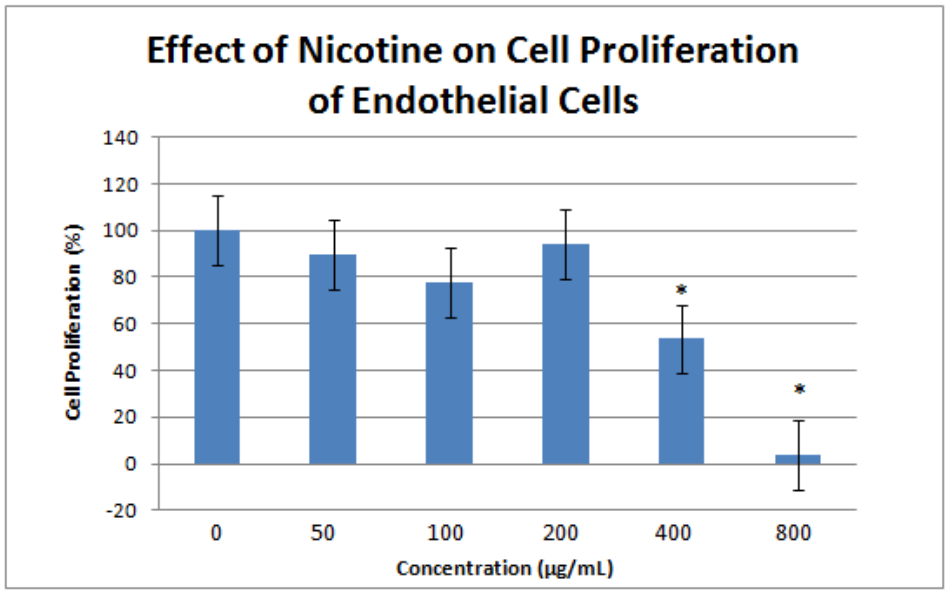


Figure 3. Effects of nicotine on endothelial cell proliferation at various concentrations. A significant decrease in cell proliferation was exhibited at 400 ($p=0.049$) and 800 ($p<0.001$) µg/mL nicotine compared to the control that did not contain nicotine. Endothelial cell proliferation decreased to 53.4 ± 0.002% at 400 µg/mL and 3.5 ± 0.002% at 800 µg/mL. * Denotes statistically significant value ($p<0.05$).

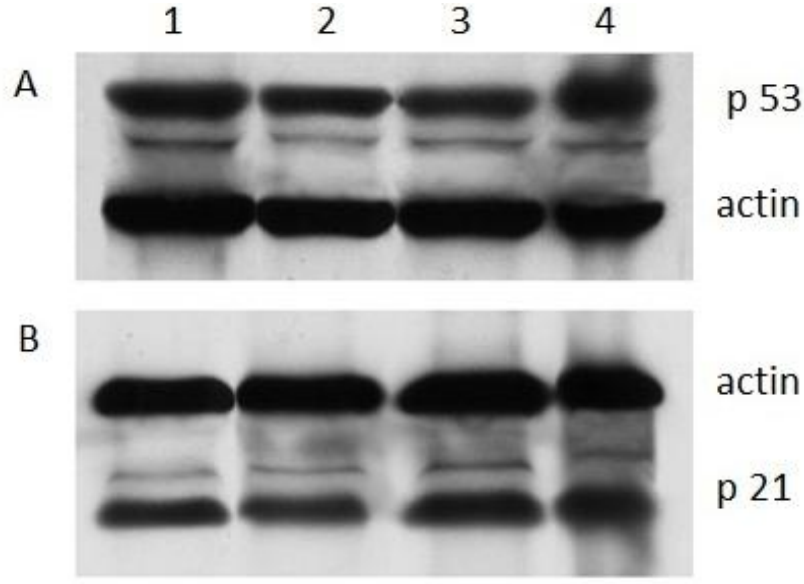


Figure 4. Effects of nicotine and CSC on p53 and p21 protein expression from HGFs Western blots probed with (A): anti-p53 and anti-actin antibodies and (B): anti-p21 and anti-actin antibodies. Lane 1: Extracts of UV-irradiated cells served as a positive control for p53 activation (cells irradiated with 20 J/m2 of UV radiation (254 nm) and then incubated for 3 h in serum-free medium). Lane 2: Untreated cells. Lane 3: cells treated with 100 µg/ml cigarette smoke condensate for 72 hours, and lane 4: cells treated with 250 µg/ml nicotine for 72 hours. Actin was used as a loading control.

Cigarette Smoke Condensate (CSC)

Cigarette smoke condensate (CSC), the particulate matter of cigarette smoke, is composed of more than 4,000 different components [91]. The particulate phase or CSC is composed of toxicants such as nicotine, phenol, anthracyclic hydrocarbons, nitrosamines, heavy metals, and chemical carcinogens including 4-(methylnitrosaminno)-1-(3-pyridyl)-1-butanone (NNK) [91]. CSC destroys the balance between the MMPs and TIMPs and this results in increased collagen degradation[92]. Cell proliferation was reduced to 46.8% when CSC concentration reached 200 µg/ml [92]. CSC at concentrations of 400 and 800 µg/ml demonstrated significant toxic effects on the HGFs [92]. When using a concentration of 50 µg/ml of CSC to treat the HGFs, the active form of MMP-2 increased more than 16-fold when compared to the control in the conditioned media, while MMP-14 increased by more than 2-fold in the membrane extracts from HGFs [92]. In regards to the TIMPs, TIMP-1 increased its level in the conditioned media of the HGFs treated with CSC [92]. TIMP-2 decreased with increasing concentrations of CSC in the conditioned media and increased with increasing concentrations of CSC in the membrane extracts [92]. When 10% of *P. gingivalis* supernatant was combined with 100 µg/ml of CSC and added to HGFs, it significantly reduced the viability of the HGFs [93]. However, when 10% of *P. gingivalis* supernatant was combined with 50 µg/ml of CSC and added to the HGFs, no significant toxic effect on the HGFs was noted [93]. The combined effect of CSC and *P. gingivalis* supernatant accelerated the collagen degradation mediated by the HGFs and increased the activation of several MMPs (e.g., MMP-1, MMP-2, and MMP-14) suggesting they may play a role in the increased collagen degradation [93].

Volatile components of cigarette smoke such as acrolein and acetaldehyde were cytopathic to gingival fibroblasts*in vitro* [94]. These components caused a dose-dependent inhibition of cell adhesion and disruption of vimentin intermediate filaments due to their ability to bind to and interact with the fibroblast's cytoskeleton. Therefore, they directly impaired the ability of the periodontal tissues to regenerate and repair damaged connective tissues.

CSC stimulated urokinase production and plasminogen activation in HGFs [95]. This CSC-stimulated urokinase production depends on both the activation of the extracellular signal-regulated kinase/c-Jun N-terminal kinase pathways and also on the generation of intracellular reactive oxygen species[95]. This can lead to alteration of the connective tissue remodeling process.

It has been demonstrated that nicotine and CSC increased p53 and p21 levels in HGFs, which suggest that tobacco induces DNA damage in the HGFs even after one exposure (Figure 4). This could result in p53/p21 inhibiting cell division, which may increase the rate of tissue destruction reported in the periodontal tissues of smokers. In addition, increased p53 expression could possibly induce apoptosis of the fibroblasts, which could contribute to impaired healing of the periodontal tissues in smokers.

Conclusion

In conclusion, tobacco usage has many effects on the cells of the oral cavity that may increase susceptibility to periodontal disease and result in the poorer responses to various treatments. In view of the data and the deleterious effects that tobacco has on the different cells of the oral cavity, smoking/tobacco cessation should be an important treatment consideration for periodontal patients as well as all patients.

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References

[1] Summers CJ, O.A., Association of oral disease with 12 selected variables: I. Periodontal disease. *Journal of Dental Research*, 1968. 47: p. 457-462.

[2] Bergstrom, J. and B. Floderus-Myrhed, Co-twin control study of the relationship between smoking and some periodontal disease factors. *Community Dentistry and Oral Epidemiology*, 1983. 11(2): p. 113-6.

[3] Feldman, R.S., J.S. Bravacos, and C.L. Rose, Association between smoking different tobacco products and periodontal disease indexes. *J. Periodontol*, 1983. 54(8): p. 481-7.

[4] Ismail, A.I., B.A. Burt, and S.A. Eklund, Epidemiologic patterns of smoking and periodontal disease in the United States. *J. Am. Dent. Assoc*, 1983. 106(5): p. 617-21.

[5] Bergstrom, J., S. Eliasson, and H. Preber, Cigarette smoking and periodontal bone loss. *J. Periodontol*, 1991. 62(4): p. 242-6.

[6] Haber, J. and R.L. Kent, Cigarette smoking in a periodontal practice. *J. Periodontol*, 1992. 63(2): p. 100-6.

[7] Tomar, S.L. and S. Asma, Smoking-attributable periodontitis in the United States: findings from NHANES III. National Health and Nutrition Examination Survey. J. *Periodontol*, 2000. 71(5): p. 743-51.

[8] Benowitz, N.L. and P. Jacob, 3rd, Daily intake of nicotine during cigarette smoking. *Clin. Pharmacol. Ther*, 1984. 35(4): p. 499-504.

[9] Kozlowski, L.T., R.C. Frecker, and H. Lei, Nicotine yields of cigarettes, plasma nicotine in smokers, and public health. *Prev. Med*, 1982. 11(2): p. 240-4.

[10] Pindborg, J.J., Tobacco and gingivitis. *J. Dent. Res*, 1947. 26(3): p. 261: passim.

[11] Tonetti, M.S., Cigarette smoking and periodontal diseases: etiology and management of disease. *Ann. Periodontol*, 1998. 3(1): p. 88-101.

[12] Gelskey, S.C., Cigarette smoking and periodontitis: methodology to assess the strength of evidence in support of a causal association. *Community Dentistry and Oral Epidemiology*, 1999. 27(1): p. 16-24.

[13] Haber, J., et al., Evidence for cigarette smoking as a major risk factor for periodontitis. *J. Periodontol*, 1993. 64(1): p. 16-23.
[14] Pindborg, J.J., Tobacco and gingivitis; correlation between consumption of tobacco, ulceromembranous gingiivitis and calculus. *J. Dent. Res*, 1949. 28(5): p. 460-3.
[15] Ainamo, J., The seeming effect of tobacco consumption on the occurrence of periodontal disease and dental caries. Suom Hammaslaak Toim, 1971. 67(2): p. 87-94.
[16] Markkanen, H., et al., Smoking and periodontal disease in the Finnish population aged 30 years and over. *J. Dent. Res*, 1985. 64(6): p. 932-5.
[17] Macgregor, I.D., W.M. Edgar, and A.R. Greenwood, Effects of cigarette smoking on the rate of plaque formation. *J. Clin. Periodontol*, 1985. 12(1): p. 35-41.
[18] Preber, H. and J. Bergstrom, Occurrence of gingival bleeding in smoker and non-smoker patients. *Acta Odontol. Scand*, 1985. 43(5): p. 315-20.
[19] Clarke, N.G., B.C. Shephard, and R.S. Hirsch, The effects of intra-arterial epinephrine and nicotine on gingival circulation. *Oral Surg. Oral Med. Oral Pathol*, 1981. 52(6): p. 577-82.
[20] Holmes, L.G., [Effects of smoking and/or vitamin C on sulcular fluid rate in clinically healthy gingiva]. Quintessenz, 1990. 41(3): p. 465-71.
[21] Shirodaria, S., et al., Polymorphisms in the IL-1A gene are correlated with levels of interleukin-1alpha protein in gingival crevicular fluid of teeth with severe periodontal disease. *J. Dent. Res*, 2000. 79(11): p. 1864-9.
[22] Haffajee, A.D. and S.S. Socransky, Relationship of cigarette smoking to attachment level profiles. *J. Clin. Periodontol*, 2001. 28(4): p. 283-95.
[23] Bergstrom, J., S. Eliasson, and J. Dock, A 10-year prospective study of tobacco smoking and periodontal health. *J. Periodontol*, 2000. 71(8): p. 1338-47.
[24] Mullally, B.H. and G.J. Linden, Molar furcation involvement associated with cigarette smoking in periodontal referrals. *J. Clin. Periodontol*, 1996. 23(7): p. 658-61.
[25] Craig, R.G., et al., Prevalence and risk indicators for destructive periodontal diseases in 3 urban American minority populations. *J. Clin. Periodontol*, 2001. 28(6): p. 524-35.
[26] Daniell, H.W., Postmenopausal tooth loss. Contributions to edentulism by osteoporosis and cigarette smoking. *Arch Intern. Med*, 1983. 143(9): p. 1678-82.
[27] McGuire, M.K. and M.E. Nunn, Prognosis versus actual outcome. IV. The effectiveness of clinical parameters and IL-1 genotype in accurately predicting prognoses and tooth survival. *J. Periodontol*, 1999. 70(1): p. 49-56.
[28] Mullally, B.H., The influence of tobacco smoking on the onset of periodontitis in young persons. *Tob. Induc. Dis*, 2004. 2(2): p. 53-65.
[29] Linden, G.J. and B.H. Mullally, Cigarette smoking and periodontal destruction in young adults. *J. Periodontol*, 1994. 65(7): p. 718-23.
[30] Kaldahl, W.B., et al., Levels of cigarette consumption and response to periodontal therapy. *J. Periodontol*, 1996. 67(7): p. 675-81.
[31] Zambon, J.J., et al., Cigarette smoking increases the risk for subgingival infection with periodontal pathogens. *J. Periodontol*, 1996. 67(10 Suppl): p. 1050-4.
[32] Martinez-Canut, P., A. Lorca, and R. Magan, Smoking and periodontal disease severity. *J. Clin. Periodontol*, 1995. 22(10): p. 743-9.
[33] Kowolik, M.J. and T. Nisbet, Smoking and acute ulcerative gingivitis. A study of 100 patients. *Br. Dent. J*, 1983. 154(8): p. 241-2.

[34] Stammers, A.F., Vincent's Ulceration: The Problem of Persistent and Recurrent Infection. *Proc. R. Soc. Med*, 1944. 37(10): p. 567-574.
[35] Murayama, Y., et al., Acute necrotizing ulcerative gingivitis: risk factors involving host defense mechanisms. *Periodontol. 2000*, 1994. 6: p. 116-24.
[36] Shannon, I.L., W.G. Kilgore, and T.J. O'Leary, Stres as a predisposing factor in necrotizing ulcerative gingivitis. *J. Periodontol*, 1969. 40(4): p. 240-2.
[37] Robertson, P.B., M.M. Walsh, and J.C. Greene, Oral effects of smokeless tobacco use by professional baseball players. *Adv. Dent. Res*, 1997. 11(3): p. 307-12.
[38] Wray, A. and W.F. McGuirt, Smokeless tobacco usage associated with oral carcinoma. Incidence, treatment, outcome. *Arch Otolaryngol. Head Neck Surg*, 1993. 119(9): p. 929-33.
[39] Van der Velden, U., et al., Effect of smoking and periodontal treatment on the subgingival microflora. *J. Clin. Periodontol*, 2003. 30(7): p. 603-10.
[40] Preber, H., J. Bergstrom, and L.E. Linder, Occurrence of periopathogens in smoker and non-smoker patients. *J. Clin. Periodontol*, 1992. 19(9 Pt 1): p. 667-71.
[41] Stoltenberg, J.L., et al., Association between cigarette smoking, bacterial pathogens, and periodontal status. *J. Periodontol*, 1993. 64(12): p. 1225-30.
[42] Darby, I.B., et al., Microbial comparison of smoker and non-smoker adult and early-onset periodontitis patients by polymerase chain reaction. *J. Clin. Periodontol*, 2000. 27(6): p. 417-24.
[43] Zitterbart, P.A., et al., Association between cigarette smoking and the prevalence of dental cariesin adult males. *Gen. Dent*, 1990. 38(6): p. 426-31.
[44] Lindemeyer, R.G., et al., In vitro effect of tobacco on the growth of oral cariogenic streptococci. *J. Am. Dent. Assoc*, 1981. 103(5): p. 719-22.
[45] Gregory, R.L., Marquie, M. A., McNutt, K., Gurdogan, E., Brown, L., Zheng, C. , Effects of tobacco on Streptococcus mutans virulence activities. Abstracts of the Society for Research on Nicotine and Tobacco. , 2009.
[46] Zheng, C., Gregory R.L., Tobacco smoking condensate affects the sucrose-dependent adherence of oral streptococci. . Abstracts of the International Association of Dental Research, 2009.
[47] Ah, M.K., et al., The effect of smoking on the response to periodontal therapy. *J. Clin. Periodontol*, 1994. 21(2): p. 91-7.
[48] Soder, B., U. Nedlich, and L.J. Jin, Longitudinal effect of non-surgical treatment and systemic metronidazole for 1 week in smokers and non-smokers with refractory periodontitis: a 5-year study. *J. Periodontol*, 1999. 70(7): p. 761-71.
[49] Palmer, R.M., et al., Potential mechanisms of susceptibility to periodontitis in tobacco smokers. *Journal of Periodontal Research*, 1999. 34(7): p. 363-9.
[50] Scabbia, A., et al., Cigarette smoking negatively affects healing response following flap debridement surgery. *J. Periodontol*, 2001. 72(1): p. 43-9.
[51] Tonetti, M.S., et al., Enamel matrix proteins in the regenerative therapy of deep intrabony defects. *J. Clin. Periodontol*, 2002. 29(4): p. 317-25.
[52] Trombelli, L. and A. Scabbia, Healing response of gingival recession defects following guided tissue regeneration procedures in smokers and non-smokers. *J. Clin. Periodontol*, 1997. 24(8): p. 529-33.

[53] Souza, S.L., et al., Subepithelial connective tissue graft for root coverage in smokers and non-smokers: a clinical and histologic controlled study in humans. *J. Periodontol,* 2008. 79(6): p. 1014-21.

[54] Rosen, P.S., M.H. Marks, and M.A. Reynolds, Influence of smoking on long-term clinical results of intrabony defects treated with regenerative therapy. *J. Periodontol,* 1996. 67(11): p. 1159-63.

[55] Luepke, P.G., J.T. Mellonig, and M.A. Brunsvold, A clinical evaluation of a bioresorbable barrier with and without decalcified freeze-dried bone allograft in the treatment of molar furcations. *J. Clin. Periodontol,* 1997. 24(6): p. 440-6.

[56] Johnson, G.K. and M. Hill, Cigarette smoking and the periodontal patient. *J. Periodontol,* 2004. 75(2): p. 196-209.

[57] Haas, R., et al., The relationship of smoking on peri-implant tissue: a retrospective study. *J. Prosthet Dent,* 1996. 76(6): p. 592-6.

[58] Bain, C.A. and P.K. Moy, The association between the failure of dental implants and cigarette smoking. *Int. J. Oral Maxillofac Implants,* 1993. 8(6): p. 609-15.

[59] Bain, C.A., Smoking and implant failure--benefits of a smoking cessation protocol. *Int. J. Oral Maxillofac Implants,* 1996. 11(6): p. 756-9.

[60] Schwartz-Arad, D., N. Samet, and A. Mamlider, Smoking and complications of endosseous dental implants. *J. Periodontol,* 2002. 73(2): p. 153-7.

[61] Kinane, D.F. and M. Radvar, The effect of smoking on mechanical and antimicrobial periodontal therapy. *J. Periodontol,* 1997. 68(5): p. 467-72.

[62] Theilig, C., et al., Reactions of human keratinocytes in vitro after application of nicotine. *Skin Pharmacol,* 1994. 7(6): p. 307-15.

[63] Johnson, G.K. and C.C. Organ, Prostaglandin E2 and interleukin-1 concentrations in nicotine-exposed oral keratinocyte cultures. *Journal of Periodontal Research,* 1997. 32(5): p. 447-54.

[64] El Ahmer, O.R., et al., The effect of cigarette smoke on adherence of respiratory pathogens to buccal epithelial cells. *FEMS Immunol. Med. Microbiol,* 1999. 23(1): p. 27-36.

[65] Giannopoulou, C., N. Roehrich, and A. Mombelli, Effect of nicotine-treated epithelial cells on the proliferation and collagen production of gingival fibroblasts. *J. Clin. Periodontol,* 2001. 28(8): p. 769-75.

[66] Gregory, R.L. and L.E. Gfell, Effect of nicotine on secretory component synthesis by secretory epithelial cells. *Clin. Diagn. Lab. Immunol,* 1996. 3(5): p. 578-83.

[67] Giannopoulou, C. and G. Cimasoni, Functional characteristics of gingival and periodontal ligament fibroblasts. *J. Dent. Res,* 1996. 75(3): p. 895-902.

[68] Visse, R. and H. Nagase, Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ. Res,* 2003. 92(8): p. 827-39.

[69] Zhou, J., B.L. Olson, and L.J. Windsor, Nicotine increases the collagen-degrading ability of human gingival fibroblasts. *Journal of Periodontal Research,* 2007. 42(3): p. 228-35.

[70] Almasri, A., et al., Nicotine and lipopolysaccharide affect cytokine expression from gingival fibroblasts. *J. Periodontol,* 2007. 78(3): p. 533-41.

[71] Peacock, M.E., et al., The effect of nicotine on reproduction and attachment of human gingival fibroblasts in vitro. *J. Periodontol,* 1993. 64(7): p. 658-65.

[72] Tanur, E., et al., Effects of nicotine on the strength of attachment of gingival fibroblasts to glass and non-diseased human root surfaces. *J. Periodontol,* 2000. 71(5): p. 717-22.
[73] Chang, Y.C., et al., Mechanisms of cytotoxicity of nicotine in human periodontal ligament fibroblast cultures in vitro. *Journal of Periodontal Research,* 2002. 37(4): p. 279-85.
[74] Giannopoulou, C., A. Geinoz, and G. Cimasoni, Effects of nicotine on periodontal ligament fibroblasts in vitro. *J. Clin. Periodontol,* 1999. 26(1): p. 49-55.
[75] Fang, Y. and K.K. Svoboda, Nicotine inhibits myofibroblast differentiation in human gingival fibroblasts. *J. Cell Biochem,* 2005. 95(6): p. 1108-19.
[76] Canakci, C.F., Y. Cicek, and V. Canakci, Reactive oxygen species and human inflammatory periodontal diseases. *Biochemistry* (Mosc), 2005. 70(6): p. 619-28.
[77] Drost, E.M., et al., Changes in neutrophil deformability following in vitro smoke exposure: mechanism and protection. *Am. J. Respir. Cell Mol Biol,* 1992. 6(3): p. 287-95.
[78] Pabst, M.J., et al., Inhibition of neutrophil and monocyte defensive functions by nicotine. *J. Periodontol,* 1995. 66(12): p. 1047-55.
[79] Aida, Y., et al., Lipopolysaccharides from periodontal pathogens prime neutrophils for enhanced respiratory burst: differential effect of a synthetic lipid a precursor IVA (LA-14-PP). *Journal of Periodontal Research,* 1995. 30(2): p. 116-23.
[80] Sorensen, L.T., et al., Effect of smoking and abstention on oxidative burst and reactivity of neutrophils and monocytes. *Surgery,* 2004. 136(5): p. 1047-53.
[81] Benhammou, K., et al., [(3)H]Nicotine binding in peripheral blood cells of smokers is correlated with the number of cigarettes smoked per day. *Neuropharmacology,* 2000. 39(13): p. 2818-29.
[82] Ackermann, M.F., et al., Selective inhibition of polymorphonuclear neutrophil activity by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicol. Appl. Pharmacol,* 1989. 101(3): p. 470-80.
[83] Lebargy, F., et al., Tobacco smoking induces expression of very-high-affinity nicotine binding sites on blood polymorphonuclear cells. *Am. J. Respir. Crit. Care Med,* 1996. 153(3): p. 1056-63.
[84] Seow, W.K., et al., Nicotine-induced release of elastase and eicosanoids by human neutrophils. *Inflammation,* 1994. 18(2): p. 119-27.
[85] Soder, B., L.J. Jin, and S. Wickholm, Granulocyte elastase, matrix metalloproteinase-8 and prostaglandin E2 in gingival crevicular fluid in matched clinical sites in smokers and non-smokers with persistent periodontitis. *J. Clin. Periodontol,* 2002. 29(5): p. 384-91.
[86] Mariggio, M.A., et al., Nicotine effects on polymorphonuclear cell apoptosis and lipopolysaccharide-induced monocyte functions. A possible role in periodontal disease? *Journal of Periodontal Research,* 2001. 36(1): p. 32-9.
[87] Sopori, M.L., et al., Effect of nicotine on the immune system: possible regulation of immune responses by central and peripheral mechanisms. *Psychoneuroendocrinology,* 1998. 23(2): p. 189-204.
[88] Sopori, M.L., et al., Cigarette smoke causes inhibition of the immune response to intratracheally administered antigens. *Toxicol. Appl. Pharmacol,* 1989. 97(3): p. 489-99.

[89] Mili, F., et al., The associations of race, cigarette smoking, and smoking cessation to measures of the immune system in middle-aged men. *Clin. Immunol. Immunopathol,* 1991. 59(2): p. 187-200.

[90] Park, Y.J., et al., Effect of nicotine on human umbilical vein endothelial cells (HUVECs) migration and angiogenesis. *Vascul Pharmacol,* 2008. 49(1): p. 32-6.

[91] Gao, S., et al., Transcriptional and posttranscriptional inhibition of lysyl oxidase expression by cigarette smoke condensate in cultured rat fetal lung fibroblasts. *Toxicol. Sci,* 2005. 87(1): p. 197-203.

[92] Zhang, W., F. Song, and L.J. Windsor, Cigarette smoke condensate affects the collagen-degrading ability of human gingival fibroblasts. *Journal of Periodontal Research,* 2009. 44(6): p. 704-13.

[93] Zhang, W., F. Song, and L.J. Windsor, Effects of tobacco and P. gingivalis on gingival fibroblasts. *J. Dent. Res,* 2010. 89(5): p. 527-31.

[94] Poggi, P., M.T. Rota, and R. Boratto, The volatile fraction of cigarette smoke induces alterations in the human gingival fibroblast cytoskeleton. *Journal of Periodontal Research,* 2002. 37(3): p. 230-5.

[95] Gonzalez, R., et al., Cigarette smoke condensate stimulates urokinase production through the generation of reactive oxygen species and activation of the mitogen activated protein kinase pathways in human gingival fibroblasts. *Journal of Periodontal Research,* 2009. 44(3): p. 386-94.

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Chapter VII

A Novel Cytodiagnostic Fluorescence Assay for the Diagnosis of Periodontitis

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Abstract

A topical issue in periodontology is to find objective diagnostic methods which may be combined with the classical clinical inspection parameters to yield a reliable grading of the severity and extent of periodontal disease. This study deals with a novel cytodiagnostic fluorescence test, performed on exfoliation samples taken from periodontal/oral tissues, useful to assess the severity of periodontal disease. Twenty-one patients with different degrees of periodontitis were subjected to clinical and histopathological grading and the results compared with those obtained from the cytodiagnostic fluorescence assay. We found that the amount of blood cells (polymorphonuclear and mononuclear leukocytes, erythrocytes), the occurrence of morphologically abnormal epithelial cells, and the number of spirochetes showed a statistically significant correlation with the clinical and histopathological diagnostic parameters, the latter being considered as the most reliable predictors of the severity of periodontal disease. On these grounds, we suggest that this cytodiagnostic method may greatly help dental practitioners to achieve a chair-side, reliable and objective evaluation of the degree and activity of periodontitis at first dental visit, and to perform a targeted treatment and an accurate follow up of the patients during supportive periodontal therapy.

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Introduction

Periodontal diseases comprise a group of inflammatory diseases of the gingival and supporting structures of the periodontium of high social impact, which, in the most severe cases, can result in tooth loss [Armitage et al. 1995; Papapanou et al. 1996]. Multiple infective agents are involved in the pathogenesis of this disease, including both Gram-negative anaerobic and facultative anaerobic bacteria [Loesche and Grossman, 2001]. In particular, it is generally assumed that Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans, Fusobacterium nucleatum, Bacteroides forsythus, Campylobacter rectus, Prevotella intermedia, and the oral treponemes Treponema denticola, Treponema pectinovorum, Treponema vincentii, Selenomonas sputigena, and Eikenella corrodens are associated with the most aggressive and destructive forms of periodontitis [Socransky et al.1998; Holt et al. 1999]. Indeed, these bacteria release multiple virulence factors, such as lipopolysaccharide (LPS), that activate the host inflammatory response, characterized by migration into the periodontal tissues and gingival sulcus of polymorphonuclear leukocytes (PMN) in the acute phase and mononuclear (MN) leucocytes, such as monocytes and lymphocytes, in the chronic phase, which then initiate alveolar bone resorption [Jiang et al. 1999]. The frequent relapses and chronicization of periodontitis, may depend on complex host-parasite interactions, including the ability of the above pathogens to gain access into the cells of the sulcular and buccal epithelia [Rudney et al. 2005, Apsey et al. 2006; Colombo et al. 2007; Savage et al. 2009], thereby escaping the traditional antiseptic therapies [Rudney et al. 2001; Eick and Pfister, 2004; Rautemaa et al. 2004; Andrian et al. 2006].

Recently, chronic periodontitis has posed a significant public –health challenge, because it has been suggested to be a risk factor for cardiovascular disease and preterm births [Khader and Ta'ani, 2005; Friedewald et al. 2009]. In particular, increased carotid artery intimal/medial thickness, evaluated echographically, which is associated with increased risk for acute myocardial infarction and stroke often occurs in patients with periodontitis, suggesting that subclinical atherosclerosis is present in periodontopathic patients [Scannapieco et al. 2003; Bahekar et al. 2007]. The pathophysiology for such association may rely on multiple interwoven mechanisms, including systemic release of pro-inflammatory mediators [Linden et al. 2008], endothelial dysfunction [Amar et al. 2003; Tonetti et al. 2007], and dissemination of Gram-negative bacteria from the periodontal reservoir to atheromasic lesions [Haynes and Stanford 2003].

Clinical attachment level (CAL), probing depth (PD), gingival recession and radiographic assessments of inflammation are the most widely accepted and used tools to diagnose periodontitis [Savage et al. 2009], although they may not closely reflect the actual disease activity at given sites [Caton et al. 1981; Apsey et al. 2006; Schatzle et al. 2009]. A major limitation of periodontal probing is its inability to distinguish previous tissue loss from current disease activity and is not a safe criterium for evaluating gingival health [de Souza et al. 2003]. Since periodontal tissue damage accumulates over time, the disease may appear more severe in elderly patients than in young ones, although in terms of disease progression, the contrary may be the case [Hujoel et al. 2005]. The evaluation of the outcome of therapy and detection of periodontal disease recurrence also suffers from the low sensitivity of the clinical diagnostic tests, often leading to false conclusions concerning the efficacy of the methods and treatments applied [Kaldahl et al. 1996; Mombelli, 2005]. To overcome these

limitations, additional diagnostic tools have being developed to diagnose and assess therapeutic efficacy, including microbiological and histopathogical tests. On the other hand, both these methods have several intrinsic limitations that hinder their widespread use in current dental practice. In fact, microbiological criteria fraught with technical problems related to the culturing of plaque samples for anaerobesand results can take weeks to be obtained [Apsey et al. 2006]. Likewise, histopathology on periodontal biopsies, which in principle may be the objective method of choice, finds limited application because is it invasive and need several days and specifically trained examiners to be carried out properly [Gillet et al. 1990]. Therefore, a topical issue in periodontology is to find objective diagnostic methods for the "chairside" determination of the oral disease which, in combination with the clinical inspection, may allow a rapid and reliable evaluation of the severity, extent and progression of periodontal disease, required for the set up of appropriate therapeutic protocols and follow up. Indeed, new diagnostic tools have been proposed, based on the use of highly sensitive immunoassays, enzymatic (BANA test) and real time PCR, which allow the identification of specific inflammatory and bacterial-derived biomarkers from the whole saliva and plaque bio-film of periodontal patients [Loeshe et al. 1990; Ramseier et al. 2009]. The present report provides evidence for the possible usefulness of a novel cytodiagnostic fluorescence test performed on exfoliation samples taken from periodontal/oral tissues to predict the severity of periodontal disease at individual sites. This approach is more sensitive compared with the clinical judgement and shows a strong positive correlation with the histopathological parameters indicative of disease activity.

Materials and Methods

Patients and Sampling

The study was designed in compliance with the guidelines of the Declaration of Helsinki, as amended in Edinburgh, 2003. It received a favourable ethical opinion from the Ethical Committee of the Faculty of Medicine, University of Florence, Italy. Twenty-one volunteer patients (14 males, 7 females , aged 35-65 years; mean, 58.4 years) with clinical diagnosis of periodontitis were enrolled. They attended a screening visit at which written informed consent was taken. One week before entering the study, the patients were subjected to supra-gingival tooth cleaning. The exclusion criteria were: systemic diseases (diabetes mellitus, cancer, HIV, metabolic and endocrine diseases), pregnancy and lactation, chronic high-dose steroid therapy, radiation or immunosuppressive therapy, smoking (more than 10 cigarettes/day), orthodontic treatments, extensive carious lesions, and antibiotic medication during or within the 6 months preceding the study. Periodontal clinical measurements were performed by an experienced dentist and included clinical attachment level (CAL), pocket depth (PD) and gingival index and bleeding on probing (GI/BOP). Measurements were carried out using a conventional manual periodontal probe (Hu-Friedy, Chigago, IL, USA) at 6 sites per tooth. Twenty-one randomly chosen sites (1 from each patient) were selected for histological and cytofluorescence monitoring . To delineate the area for histological analysis, a reference small incision was made on the facial surface on the gingiva which corresponded to the depth and mesio-distal extent of the area probed and evaluated for visual signs of inflammation.All sites

were isolated with cotton rolls and the supra-gingival biofilm was carefully removed with a sterile gauze. Samples of exfoliated cells were taken with a sterile micro-curette in the proximity of the free gingival margin, i.e. in the same location where histological sampling was scheduled. The samples were processed for fluorescent staining as described below. Small gingival biopsies, about 2 mm^3, were taken under local anesthesia (Articain HCl, Ultracain,, Frankfurt, Germany) from each cytological sites using a biopsy punch, 2-mm diameter, taking care not to expose the marginal alveolar bone and periostium, and routinely processed as described below.

Histological Analysis

The periodontal biopsies were immediately fixed by immersion in 4% (w/v formaldehyde in 0.2 M phosphate-buffered saline, pH 7.4, dehydrated in graded ethanol and embedded in paraffin. Five μm-thick cross sections were stained with hematoxylin and eosin, viewed and photographed under a light microscope (Nikon, Tokyo, Japan).

Table 1. Severity scoring criteria

SCORE	0	1	2	3
CAL	-	< 2 mm	2-5 mm	> 5 mm
PD	-	< 3 mm	3-6 mm	> 6 mm
GI	normal periodontum	slight erythema slight oedema no bleeding on probing	mild erythema mild oedema with smooth gingival surface bleeding on probing	severe erythema severe oedema and ulcers spontaneous bleeding
II	absent	slight, perivascular and scattered intraepithelial	mild, continuous in the lamina propria and intraepithelial	severe, continuous in the lamina propria and dense intra-epithelial
DE	normal epithelium	outer layer shedding	outer layer shedding cell swelling	as in # 2, plus ulceration
PMN, MN, RBC	absent	< 5	5-10	> 10
DEC	normal	aberrant shape	plasma membrane rupture vacuolation	conglutination, vacuolation cell debris
cocci, bacilli, spirochetes	absent	< 10	10-30	> 30

CAL, clinical attachment level; PD, probing depth; GI, gingival index; II, inflammatory infiltrate; DE, damaged epithelium; PMN, polymorphonuclear leukocytes; MN, mononuclear leukocytes; RBC, erythrocytes; DEC, damaged epithelial cells.

Table 2. Severity scoring of periodontitis

Case No.	Clinical score				Bioptic score			Cytodiagnostic fluorescence score						
	CAL	PD	GI	Total score	II	DE	Total score	PMN	MN	RBC	DEC	Cocci	Bacilli	Spiro-chete
1	1	2	1	4	2	1	3	2	1	0	0	1	1	0
2	1	1	0	2	0	0	0	0	0	0	0	0	2	0
3	3	3	2	8	3	1	4	1	3	0	1	1	0	0
4	2	2	2	6	2	2	4	2	2	3	0	1	1	0
5	3	3	3	9	3	3	6	3	3	1	3	1	2	3
6	3	3	3	9	3	3	6	3	3	2	3	1	2	1
7	3	3	3	9	3	3	6	3	3	1	1	1	1	1
8	3	3	3	9	3	3	6	3	3	2	3	3	2	1
9	3	2	0	5	2	1	3	1	2	0	1	2	2	0
10	2	1	0	3	2	1	3	1	2	0	1	2	1	0
11	2	2	3	7	3	3	6	3	1	3	2	3	3	3
12	1	1	0	2	2	0	2	0	2	0	0	2	0	0
13	2	2	0	4	2	1	3	1	2	0	1	2	0	0
14	1	2	0	3	1	1	2	1	1	0	1	2	1	0
15	2	3	3	8	3	3	6	3	1	2	3	1	3	1

Table 2. (Continued)

Case No.	Clinical score				Bioptic score			Cytodiagnostic fluorescence score						
	CAL	PD	GI	Total score	II	DE	Total score	PMN	MN	RBC	DEC	Cocci	Bacilli	Spiro-chete
16	2	2	3	7	3	3	6	3	1	3	3	1	1	3
17	3	3	0	6	3	1	4	1	3	0	0	2	2	1
18	3	2	1	6	2	1	4	1	2	0	1	2	2	0
19	3	1	0	4	0	0	0	0	0	0	0	3	0	0
20	2	2	3	7	3	3	6	3	1	2	3	1	3	2
21	2	2	2	6	3	3	6	3	3	1	3	3	0	0

CAL, clinical attachment level; PD, probing depth; GI, gingival index; II, inflammatory infiltrate; DE, damaged epithelium; PMN, polymorphonuclear leukocytes; MN, mononuclear leukocytes; RBC, erythrocytes; DEC, damaged epithelial cells.

Table 3. Spearman's non-parametric correlation test

	vs.	*r*	*r squared*	*p* value
Clinical score	II (biopsy)	0.7670	0.5882	<0.0001
	PMN	0.8327	Gaussian approx.	<0.0001
	MN	0.5248	0.2754	0.0146
	RBC	0.6131	0.3759	0.0031
	EDC	0.6993	0.489	0.0004
	cocci	-0.07588	0.005758	0.7437 (n.s.)
	bacilli	0.4263	0.1817	0.0540 (n.s.)
	spirochetes	0.6012	0.3615	0.0039

A.

	vs.	*r*	*r squared*	*p* value
Inflammatory infiltrate (biopsy)	PMN	0,8076	Gaussian approx.	<0.0001
	MNC	0,6629	0,4395	0.0011
	RBC	0,5084	0,2584	0.0186
	EDC	0,6465	0,418	0.0015
	cocci	0	0	1 (n.s.)
	bacilli	0,2919	0,08523	0.1991 (n.s.)
	spirochetes	0,5477	0,3	0.0102

B.

II, inflammatory infiltrate; PMN, polymorphonuclear leukocytes; MN, mononuclear leukocytes; RBC, erythrocytes; EDC epithelial damage, cytological.

Cytodiagnostic Fluorescence

The LIVE/DEAD BacLight™ bacterial viability kit (Invitrogen Molecular Probes, Milan, Italy) is originally developed as an easy-to-use method for monitoring the viability of micro-organisms as a function of the membrane integrity of bacteria [Boulos et al. 1999, Berney et al. 2007, Tomàs et al. 2009]. It uses a mixture of SYTO 9 green-fluorescent and propidium iodide red-fluorescent nucleic acid stains. These stains differ both in their spectral characteristics and in their ability to penetrate healthy bacterial cells. When used alone, SYTO 9 labels all bacteria in a population — those with intact membranes and those with damaged membranes. In contrast, propidium iodide penetrates only bacteria with damaged membranes, causing a reduction in SYTO 9 fluorescence when both dyes are present. Therefore, cells with a compromised membrane, considered dead or dying, stain red, whereas cells with an intact membrane stain green. In addition, this staining method can offer a broader range of diagnostic information, such as the occurrence of inflammatory PMN and MN leukocytes, erythrocytes, and the morphological alteration of the exfoliated epithelial cells. Briefly, the collected material was smeared on a histological slide, fixed in 90% ethanol, air-dried and stained with 1 ml of the fluorescent dye solution for 2 min at 37° C. After thorough rinsing in distilled water, the samples were mounted in oil and immediately observed under a Leica 4000B fluorescent microscope (Leica Microsystems, Milan, Italy).

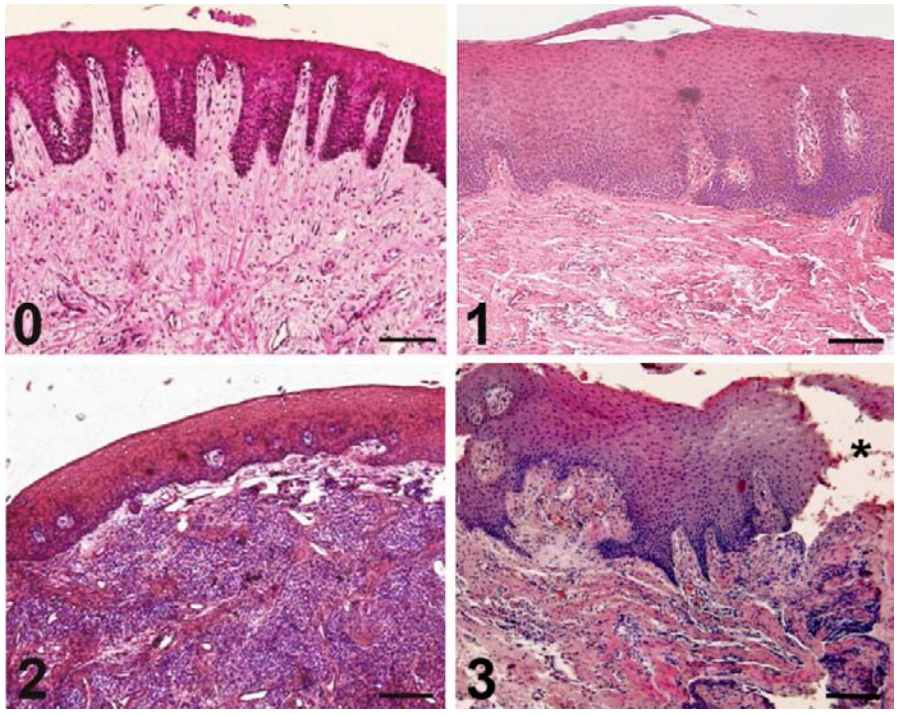

Figure 1. Histopathological scoring. Representative images of periodontal biopsies scored as indicated in Table 1: 0, normal mucosa; 1, moderate perivascular inflammatory infiltrate; 2, dense inflammatory infiltrate and reduction of epithelial cristae; 3, diffuse inflammatory infiltrate, dilated blood vessels, epithelial ulceration (asterisk). Bars = 200 μm.

Data Analysis and Statistical Evaluation

A semi-quantitative scoring was used to evaluate the severity of periodontitis based on current clinical and histopathological criteria. A similar scoring was used for the cytodiagnostic fluorescence parameters. For evaluation of bacterial score, only viable, green fluorescent stained bacteria were considered. Details are reported in Table 1. Individual patientswere assigned a total clinical and bioptic score, calculated as the sum of the scores of each clinical and histopathological parameters, respectively. The complete semi-quantitative data are reported in Table 2.

Statistical analysis of the relationships between the assayed scoring criteria was performed by the Spearman's non-parametric correlation test, assuming $p<0.05$ as statistically significant. Calculations were carried out using Prism 4.0 statistical software (Graph Pad Software, San Diego, CA).

Results

The patients under study were divided into 4 groups based on the assigned score for the clinical, histopathological and cytodiagnostic parameters (Table 2). Using the Spearman's non-parametric statistical correlation analysis, we first found a significant, positive correlation

between the clinical severity and the histopathological parameters (Table 3A). As expected, the most severe clinical signs, e.g. epithelial ulceration, spontaneous bleeding and wide dental root exposure, were associated with surface epithelial thinning and shedding, as well as with abundant inflammatory infiltrate invading the lamina propria and the epithelium in the matched bioptic samples (Figure 1)

Most of the assayed cytodiagnostic fluorescence parameters also positively correlated with the clinical severity of the periodontal disease (Table 3A), the number of PMN leukocytes and the occurrence of damaged exfoliated epithelial cells (i.e. showing spiked profile, cytoplasmic shrinkage and plasma membrane rupture) being those with the highest significance values (Figures 2, 3).

Regarding the presence of micro-organisms in the exfoliative samples (Figure 4), no significant correlation was detected between the clinical score and the number of viable cocci and bacilli within the exfoliative material - both extracellular or internalized into the epithelial cells - suggesting that their presence cannot be taken as an indicator of the active disease stage. By contrast, the presence of viable spirochetes (Figure 4) was consistently associated with severe periodontitis.

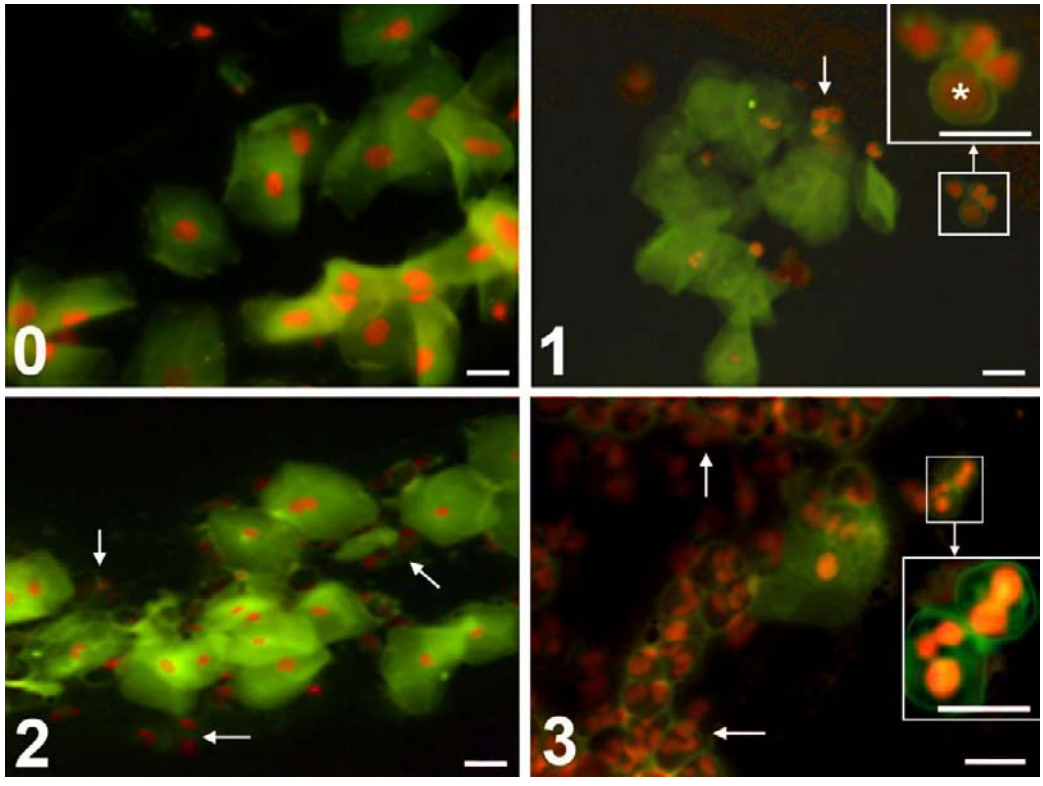

Figure 2. Cytodiagnostic fluorescence scoring. Representative images of cytological smears stained with LIVE/DEAD BacLightTM, scored as indicated in Table 1: 0, normal epithelial cells; 1, clustered epithelial cells with scattered PMN and a MN (asterisk); 2, clustered epithelial cells with several PMN; 3, a vacuolated epithelial cell with very numerous PMN. The insets show details of the leukocytes. Some PMN are indicated with arrows. Bars = 20 μm.

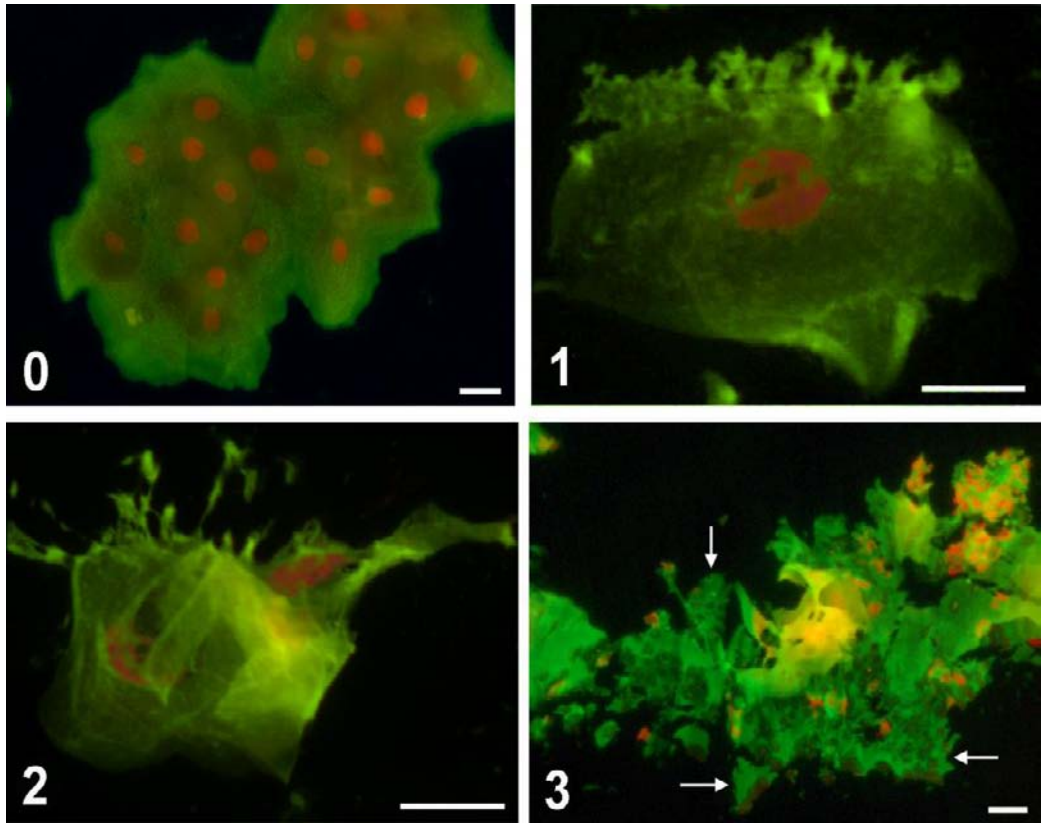

Figure 3. Cytodiagnostic fluorescence scoring. Representative images of cytological smears stained with LIVE/DEAD BacLight™, scored as indicated in Table 1: 0, normal epithelial cells; 1, an epithelial cell with spiked profile; 2, an epithelial cell with cytoplasmic shrinkage and vacuolization; 3, clustered epithelial cells with severe cytoplasmic shrinkage and vacuolization surrounded by cell debris (arrows). Bars = 10 μm.

We then compared the bioptic score, assumed as an objective measurement of the degree of periodontitis, with the cytodiagnostic parameters (Table 3B). Remarkably, the results showed that all the assayed parameters, except for the number of cocci and bacilli, correlated closely with the bioptic score. These findings indicated that the presence and amount of PMN, MN, RBC, DEC, and spirochetes in the cytodiagnostic samples had a diagnostic value comparable to that of conventional tissue biopsy.

Of note, some patients (for instances 10,12, 13, 19) diagnosed as having mild periodontal disease based upon the high CAL and PD scores, resulted either negative or positive for substantial inflammatory response and pathogenic bacterial colonization at both the biopsy and cytodiagnostic analyses. These findings, suggested that the mere clinical inspection was insufficient to correctly diagnose the periodontal disease and that its combination with the cytodiagnostic test, which displayed the same sensitivity as the histopathological one, could be essential to properly and rapidly recognize the periodontal process and address the therapeutic needs.

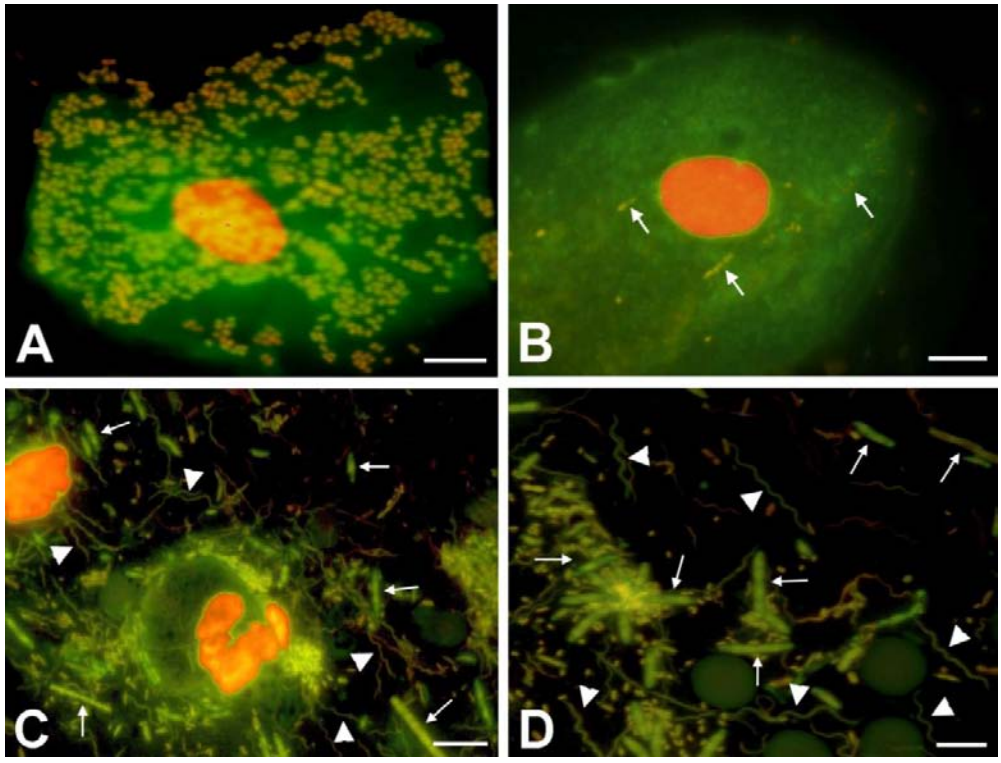

Figure 4. Cytodiagnostic fluorescence scoring. Representative images of typical oral bacteria in the cytological smears, as they appear upon staining with LIVE/DEAD BacLight™: A, cocci; adherent to and inside an epithelial cell; B, bacilli within an epithelial cell (arrows); C,D, bacilli and spirochetes within the exfoliative material. Arrows: cocci; arrowheads: spirochetes. Green and orange/red indicate viable and dead bacteria, respectively. Bars = 10 μm.

Conclusion

A serious concern in periodontology is the lack of reliable criteria to objectively distinguish between sites with active and inactive disease and differentiate between changes associated with aging and changes associated with disease. The traditional clinical parameters, such as CAL, PD and GI/BOP, as well as the assessment of dental plaque and calculus, namely tooth mobility, and radiological appearance of alveolar bone, are considered inappropriate *per se* to provide accurate diagnostic aids [Savage et al. 2009]. The present study introduces a novel cytodiagnostic method that shows a strong positive correlation with the histopathological grading. Since the histological evaluation of surgical biopsies from affected tissues is considered the best diagnostic tool to recognize the phases of periodontal disease and to differentiate the periods of destructive inflammatory response from those of effective host defense [Gillett et al. 1990], we suggest that this newly identified method, in a similar manner as histopathology but in a shorter time, may objectively reflect the activity of periodontal disease and contribute to reduce the misclassification of subjects based on the clinical evidence.

We found that the amount of blood cells, especially PMN and MN, within the exfoliated samples could be considered as reliable indicators of the degree of the periodontal inflammatory reaction, in full agreement with previous studies showing that the presence of white blood cells in the dental plaque is diagnostic for periodontal disease [Apsey et al. 2006; Vitkov et al. 2009]. This method also allowed the examiner to easily discriminate between PMN and MC, which are characteristic of the acute and chronic inflammatory phases, respectively, thus potentially providing information on the evolution of periodontitis and the efficacy of the therapy. The presence of RBC also had diagnostic value, as it was conceivably related to clinical or sub-clinical gingival bleeding.

It is worth noting that the occurrence of exfoliated epithelial cells with morphological alterations (DEC) was also correlated with the clinical and histopathological grading of periodontitis, suggesting that DEC may be a valuable diagnostic parameter of periodontitis. This assumption is in line with the well accepted role for periodontal epithelium in health and disease, providing a physical barrier to infection and participating actively in the innate host defense [Holt et al. 1999; Andrian et al. 2006; Dale 2002]. In particular, it has been demonstrated that periodontal epithelial cells respond to bacteria in an interactive manner; they secrete interleukin-8 and other chemokines and cytokines and produce natural antimicrobial peptides in response to bacterial plaque [Weinberg et al. 1998; Dale 2002; Laurina et al. 2009; Ren et al. 2009]. These cells may even respond to bacteria by changes in cell proliferation, differentiation and death, thus altering epithelial tissue homeostasis. All these data, combined with the present findings concerning the occurrence of damaged epithelial cells in the exfoliated samples of patients with periodontitis, invite dentists and researchers to pay more attention to the modifications of the periodontal epithelium and their connection with the initiation and progression of periodontal disease.

We have also demonstrated that this cytodiagnostic method can easily detect the presence of micro-organisms within the exfoliated material and, most importantly, it is able to quantify bacterial viability in real-time. Recent compelling evidence indicates that periodontal bacteria can establish symbiotic or parasitic relationships with the host depending on their ability to settle in appropriate ecological niches, such as gingival epithelial cells or fibroblasts, and produce virulence factors, affording them a competitive advantage over the commensal microbiota and resistance to host immunologic defenses [Holt et al. 1999]. Notably, this method was also able to distinguish between commensal and parodontopathic bacteria. Given that spirochetes, such as *Treponema denticola,* are considered the most aggressive periodontal pathogens [Holt et al. 1999; Colombo et al. 2007], their presence in subgingival plaque samples being associated with clinical parameters of periodontitis, such as PD and GI/BOP [Byrne et al. 2009], the cytodiagnostic fluorescence method reported in this study may help identifying sites at risk for progression and assist in the targeted treatment of periodontitis.Moreover, compared with conventional phase contrast microscopy, the present method has the advantage of distinguishing between dead and viable bacteria, thus allowing to discriminate inactive and active pathogens. At the opposite with spirochetes, the presence of cocci and bacilli within the exfoliated epithelial cells did not show any significant correlation with the degree of periodontitis: this paradox may be explained by the fact that these morphologically identified classes of micro-organisms include both active and quiescent parodontopathogens, such as for instance *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans,* as well as normal components of the oral microbial flora.

In conclusion, the current cytodiagnostic fluorescent method allows the identification of key morpho-pathological features in the exfoliation periodontal samples that strongly correlate with periodontitis. We suggest that this approach may have significant potential in assisting dental practitioners to achieve a chairside reliable and objective evaluation of the degree of the periodontal disease at the first observation and to correctly classify the patients as needing or not needing treatment. It may also represent an useful tool to perform an accurate follow up of the patients during supportive periodontal therapy. Studies are ongoing to apply this approach to the longitudinal predictions of disease activity. The development of a simple and rapid diagnostic method for periodontitis may also yield obvious benefits to reduce the related long-term cardiovascular risks and health care costs.

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References

Amar, S.; Gokce, N.; Morgan, S.; Loukideli, M.; Van Dyke, T.E.; Vita, J.A. (2003). Periodontal disease is associated with brachial artery endothelial dysfunction and systemic inflammation. *Arterioscler. Thromb. Vasc. Biol.* 23, 1245-1249.

Andrian, E.; Grenier, D.; Rouabhia, M. (2006). Porphyromonas gingivalis-epithelial cell interactions in periodontitis. *J. Dent. Res*, 85, 392-403.

Apsey, D.J.; Kaciroti, N.; Loesche, W.J. (2006). The diagnosis of periodontal disease in private practice. *J. Periodontol*, 77, 1572-1581.

Armitage, G.C. (1995). Clinical evaluation of periodontal diseases. *Periodontol*.2000, 7, 39-53.

Bahekar, A.A.; Singh, S.; Saha, S.; Molnar, J.; Arora, R. (2007). The prevalence and incidence of coronary heart disease is significantly increased in periodontitis: a meta-analysis. *Am. Heart J*, 154, 830-837.

Berney, M.; Hammes, F.; Bosshard, F.; Weilenmann, H.U.; Egli, T. (2007). Assessment and interpretation of bacterial viability by using the LIVE/DEAD BacLight Kit in combination with flow cytometry. *Appl. Environ. Mental. Microbiol*, 73, 3283-3290.

Boulos, L. ; Prévost, M.; Barbeau, B. ; Coallier, J. ; Desjardins, R. (1999). LIVE/DEAD BacLight : application of a new rapid staining method for direct enumeration of viable and total bacteria in drinking water. *J. Microbiol. Methods*, 37, 77-86.

Byrne, S.J.; Dashper, S.G.; Darby, I.B.; Adams, G.G.; Hoffmann, B.; Reynolds, E.C. (2009). Progression of chronic periodontitis can be predicted by the levels of *Porphyromonas gingivalis* and *Treponema denticola* in subgingival plaque. *Oral Microbiol. Immunol*, 24, 469-477.

Caton, J.; Greenstein, G.; Polson, A.M. (1981). Depth of periodontal probe penetration related to clinical and histologic signs of gingival inflammation. *J. Periodontol*,52, 626-629.

Colombo, A.V.; da Silva, C.M.; Haffajee, A.; Colombo, A.P. (2007). Identification of intracellular oral species within human crevicular epithelial cells from subjects with chronic periodontitis by fluorescence in situ hybridization. *J. Periodont. Res*, 42, 236-243.

Dale, B.A. (2002). Periodontal epithelium: a newly recognized role in health and disease. *Periodontol.* 2000, 30, 70-78.

Eick, S.; Pfister, W. (2004). Efficacy of antibiotics against periodontopathogenic bacteria within epithelial cells: an in vitro study. *J. Periodontol*, 75, 1327-1334.

Friedewald, V.E.; Kornman, K.S.; Beck, J.D.; Genco, R.; Goldfine, A.; Libby, P.; Offenbacher, S.; Ridker, P.M.; Van Dyke, T.E.; Roberts, W.C. (2009). The American Journal of Cardiology and Journal of Periodontology editors' consensus: periodontitis and atherosclerotic cardiovascular disease. *J. Periodontol*, 80, 1021-1032.

Gillett, I.R.; Johnson, N.W.; Curtis, M.A.; Griffiths, G.S.; Sterne, J.A.; Carman, R.J.; Bampton, J.L.; Wilton, J.M. (1990). The role of histopathology in the diagnosis and prognosis of periodontal diseases. *J. Clin. Periodontol*, 17, 673-684.

Haynes, W.G.; Stanford, C. (2003). Periodontal disease and atherosclerosis: from dental to arterial plaque. *Arterioscler. Thromb. Vasc. Biol*, 23, 1309-1311.

Holt, S.C.; Kesavalu, L.; Walker, S.; Genco, C. (1999). Virulence factors of *Porphyromonas gingivalis*. *Periodontol.* 2000, 20, 168-238.

Hujoel, P.P.; Cunha-Cruz, J.; Selipsky, H.; Saver, B.G. (2005). Abnormal pocket depth and gingival recession as distinct phenotypes. *Periodontol.* 2000, 39, 22-29.

Jiang, Y.; Graves, D.T. (1999). Periodontal pathogens stimulate CC-chemokine production by mononuclear and bone-derived cells. *J. Periodontol*, 70, 1472-1478.

Kaldahl, W.B.; Kalkwarf, K.L.; Patil, K.D.; Molvar, M.P.; Dyer, J.K. (1996). Long-term evaluation of periodontal therapy. I. Response to 4 therapeutic modalities. *J. Periodontol*, 67, 93–102.

Khader, Y.S.; Ta'ani, Q. (2005). Periodontal diseases and the risk of preterm birth and low birth weight: a meta-analysis. *J. Periodontol*, 76, 161-165.

Laurina, Z.; Pilmane, M.; Care, R. (2009). Growth factors/cytokines/defensins and apoptosis in periodontal pathologies. *Stomatologija*, 11, 48-54.

Linden, G.J.; Mcclean, K.; Young, I.; Evans, A.; Kee, F. (2008). Persistently raised C-reactive protein levels are associated with advanced periodontal disease. *J. Clin. Periodontol*,35, 741-747.

Loesche, W.J.; Grossman, N.S. (2001). Periodontal disease as a specific; albeit chronic; infection: diagnosis and treatment. *Clin. Microbiol. Rev*, 14, 727-752.

Loeshe, W.J.; Giordano, J.; Hujoel, P.P. (1990). The Utility of the BANA Test for Monitoring Anaerobic Infections due to Spirochetes (*Treponema denticola*) in Periodontal Disease. *J. Dent. Res*, 69, 1696-1702.

Mombelli, A. (2005). Clinical parameters: biological validity and clinical utility. *Periodontol*. 2000, 39, 30-39.

Papapanou, P.N. (1996).Periodontal diseases: epidemiology. *Ann. Periodontol*, 1, 1-36.

Ramseier, C.A.; Kinney, J.S.; Herr, A.E.; Braun, T.; Sugai, J.V.; Shelburne, C.A.; Rayburn, L.A.; Tran, H.M.; Singh, A.K.; Giannobile, W.V. (2009). Identification of pathogen and host-response markers correlated with periodontal disease. *J. Periodontol*, 80, 436-446.

Rautemaa, R.; Järvensivu, A.; Kari, K.; Wahlgren, J.; DeCarlo, A.; Richardson, M.; Sorsa, T. (2004). Intracellular localization of Porphyromonas gingivalis thiol proteinase in periodontal tissues of chronic periodontitis patients. *Oral Dis*, 10, 298-305.

Ren, L.; Jiang, Z.Q.; Fu, Y.; Leung, W.K.; Jin, L. (2009). The interplay of lipopolysaccharide-binding protein and cytokines in periodontal health and disease. J Clin Periodontol, 36, 619-626.

Rudney, J.D.; Chen, R.; Sedgewick, G.J. (2005). *Actinobacillus actinomycetemcomitans; Porphyromonas gingivalis; and Tannerella forsythensis* are components of a polymicrobial intracellular flora within human buccal cells. *J. Dent. Res*, 84, 59-63.

Rudney, J.D.; Chen, R.; Sedgewick, G.J. (2001). Intracellular *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in buccal epithelial cells collected from human subjects. *Infect. Immun*, 69, 2700-2707.

Savage, A.; Eaton, K.A.; Moles, D.R.; Needleman, I. (2009). A systematic review of definitions of periodontitis and methods that have been used to identify this disease. *J. Clin. Periodontol*, 36, 458-467.

Scannapieco, F.A.; Bush, R.B.; Paju, S. (2003). Associations between periodontal disease and risk for atherosclerosis; cardiovascular disease; and stroke. A systematic review. *Ann. Periodontol*, 8, 38-53.

Schätzle, M.; Faddy, M.J.; Cullinan, M.P.; Seymour, G.J.; Lang, N.P.; Bürgin, W.; Anerud, A.; Boysen, H.; Löe, H. (2009). The clinical course of chronic periodontitis: V. Predictive factors in periodontal disease. *J. Clin. Periodontol*, 36, 365-371.

Socransky ,S.S.; Haffajee, A.D.; Cugini, M.A.; Smith, C.; Kent, R.L. Jr. (1998). Microbial complexes in subgingival plaque. *J. Clin. Periodontol*, 25, 134-144.

de Souza. P.H.; de Toledo, B.E.; Rapp, G.E.; Zuza, E.P.; Neto, C.B.; Mendes, A.J. (2003). Reliability of bleeding and non-bleeding on probing to gingival histological features. *J. Int. Acad. Periodontol*, 5, 71-76.

Tomás, I.; García-Caballero, L.; Cousido, M.C.; Limeres, J.; Alvarez, M.; Diz, P. (2009). Evaluation of chlorhexidine substantivity on salivary flora by epifluorescence microscopy. *Oral Dis*, 15, 428-433.

Tonetti, M.S.; D'Aiuto, F.; Nibali, L.; Donald, A.; Storry, C.; Parkar, M.; Suvan, J.; Hingorani, A.D.; Vallance, P.; Deanfield, J. (2007). Treatment of periodontitis and endothelial function. *N. Engl. J. Med*, 356, 911-920.

Vitkov, L.; Klappacher, M.; Hannig, M.; Krautgartner ,WD. (2009). Extracellular neutrophil traps in periodontitis. *J. Periodont. Res*, 44, 664-672.

Weinberg, A.; Krisanaprakornkit, S.; Dale, B.A. (1998). Epithelial antimicrobial peptides: review and significance for oral applications. *Crit. Rev. Oral Biol. Med*, 9, 399–414.

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Chapter VIII

The Healthy Periodontium, the Diseased Periodontium

Leena Palomo and Nabil Bissada

Differentiation of health from disease is central to understanding diagnosis and treatment of periodontal diseases. It is logical to begin with an in-depth examination of the structure and physiology of the healthy periodontium.

Figure 1.

The tissues which surround and support a tooth in the jaw bones are considered to be part of the periodontium. The main function of the periodontium is to attach the tooth to the bone tissue of the jaws and to maintain the integrity of the surface of the masticatory mucosa of the oral cavity. These supportive tissues include the gingiva, cementum, periodontal ligament and alveolar bone. Upon clinical examination, the gingva is easily visible. Figure 1 Recently, increased interest in esthetics and the popularity of reconstructive procedures has repopularized the study of gingival and its parts. Figure 2. Gingiva is covered with stratified squamous epithelium. Oral epithelium, which faces the oral cavity and extends from the gingival margin to the mucogingival junction covers the clinically visible part of the free and attached gingiva. Keratinocytes make up the majority of the epithelial cells. They are characterized by their ability to produce cytoplasmic keratin filaments. Surface epithelial cells are constantly renewed with about a 10 day period required for a new cell to make its way through the epithelium and reach the stratum corneum. This interval is important in understanding wound healing and is called turnover time. New keratinocytes change as they traverse from basal cell layer towards the surface. Keratinocytes have characteristic shape depending on their position in the epithelium. In the germinative layer, the stratum basale the basal cells are attached to the basement membrane and are cuboidal or columnar. The spinous cells in the middle of the epithelium are the largest cells and have angular shape. The cells of the granular layer and superficial layer are flattened.

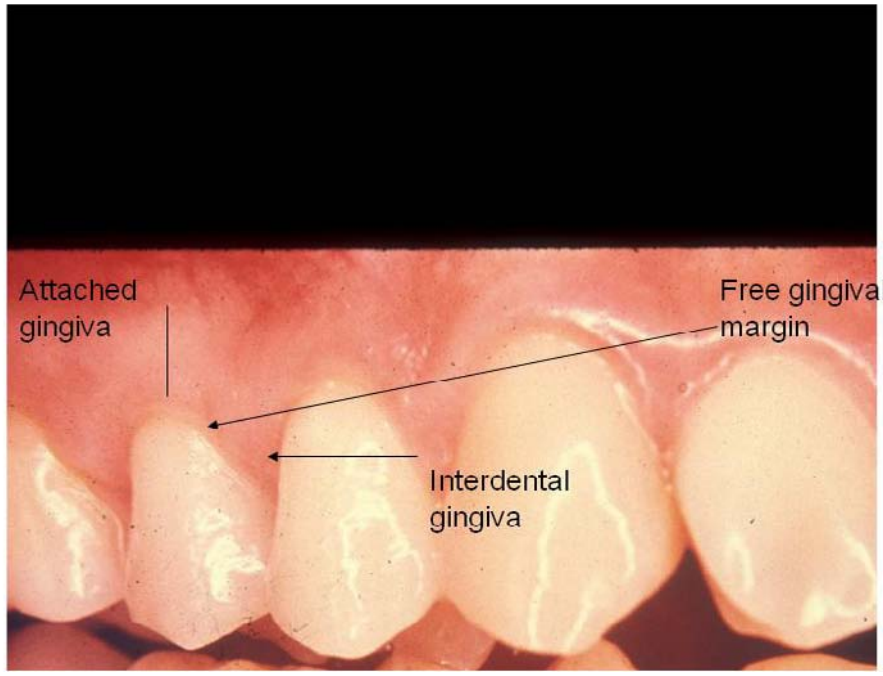


Figure 2.

The gingival sulcular epithelium extends from the oral epithelium to the gingival sulcus. This epithelium forms the wall of the gingival sulcus. It is not keratinized and may be particularly susceptible to bacterial plaque biofim. Figure 3.

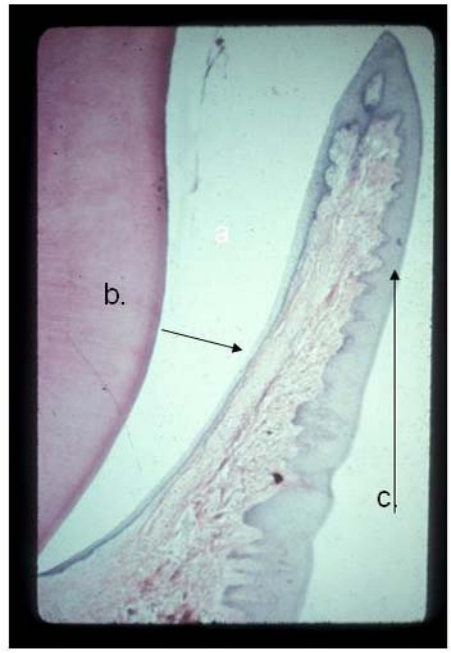


Figure 3.

Figure 4.

The junctional epithelium forms the dento-gingival junction. Figure 4. It is adapted for adherence to the tooth. It is thinner at its apical most extent, only a few cell layers thick and at thickest only 15-30 cell layers. The suprabasal cells are flat and nonkeratinized. The junctional epithelium has two basal laminas, one is attached to the connective tissueand the

other is attached to the tooth. The width also varies greatly between individuals. The free marginal gingiva varies in width from .5-2.0 and follows the scalloped contour pattern of the cemento-enamel junctions (CEJs). The color of healthy gingiva may vary according to melanogenesis, degree of keratinization, epithelial thickness and arrangement of gingival vascularity. The interdental gingiva, the interdental papilla, is often discussed in reference to anterior dental implant and reconstructive situations. Under normal, healthy conditions, it has a pyramidal or conical shape. There are two papillae, one on the facial aspect and the other on the lingual joined by a saddle-like depression, the col. Figure 5. The shape of the col depends on the location and extent of the contact area of the proximal tooth surfaces and on the height and morphology of underlying bone. (Tarnow) Due to the relationship between the native bone and the tooth contact points in the incisor region, the col may be minimal or may not even be present. The col tends to be more prominent in the molar areas. When viewed from a facial aspect the interdental papillae appear triangular in shape and when viewed from the proximal aspect appear to be concave. The esthetic importance of maintaining the form of the interdental papilla and col have led to the evolution of preservation techniques in modern periodontal plastic surgical procedures. Attached gingiva consists of a stippled tissue tightly bound to underlyng bone and cementum. Its width varies from 1-9mm. The widest area is in the facial maxillary incisor region and the narrowest is found in the buccal first premolar region. Figure 6.

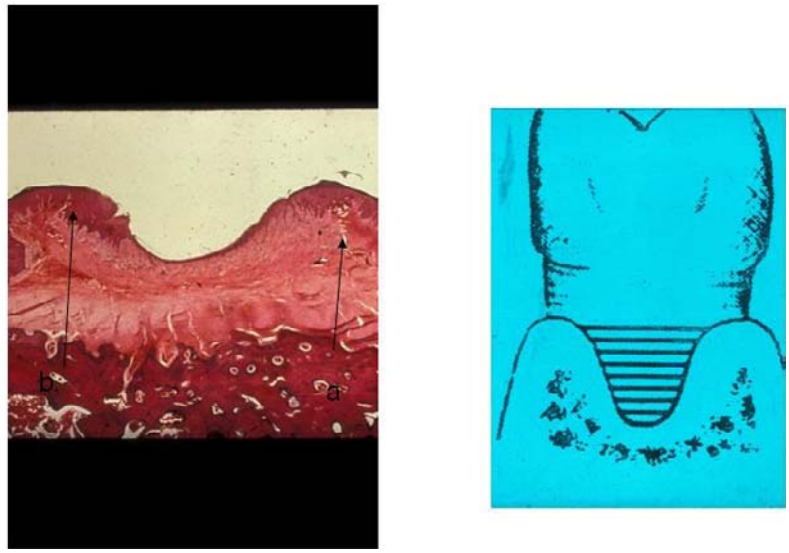


Figure 5.

Microanatomy of the periodontium is important to understand if the clinician is to take full advantage of the treatment available in modern periodontics. Gingiva is covered with stratified squamous epithelium. Oral epithelium, extends from the gingival margin to the mucogingival junction overs the clinically visible part of the free and attached gingiva. Keratinocytes make up the majority of the epithelial cells. They are characterized by their ability to produce cytoplasmic keratin filaments. Surface epithelial cells are constantly renewed.

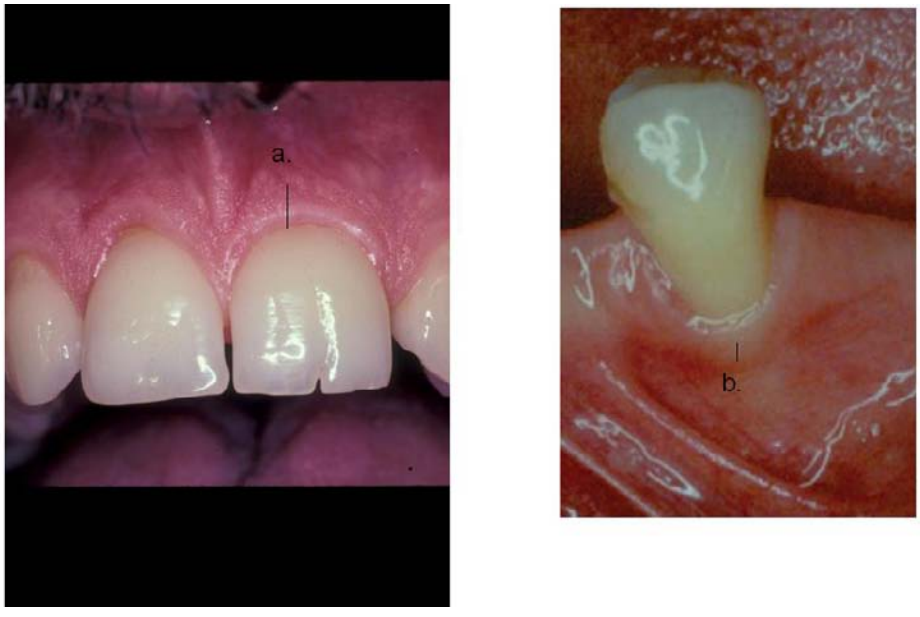


Figure 6.

Figure 7.

The cementum is a specialized calicifed tissue which covers the root surface. Because of the increased predictability and increasing popularity of regenerative procedures in modern periodontics, understanding of the properties of cementum has become very relevant. Cementum functions to attach the periodontal ligament fibers to the root and contributes to the process of repair after root surface damage. Unlike bone tissue it contains no blood or lymph vessels, has no innervation, and does not remodel. It is continuously deposited throughout life. Cementum can be fibrilar or afibrillar. Fibrilar cementum has three types: acellular, (primary), cellular, (secondary), and intermediate. Cementum is composed of 45-50% inorganic Ca2Po4 (hydroxyapatite) and 50-55% organic (collagen and water). At the cemento-enamel junction, the cementum may overlap the enamel, abut the enamel, or make no contact with it. Another function of cementum is to compensate for tooth eruption in cases of enamel loss at the occlusal surface. In the coronal third the thickness of cementum is 16-60 microns, but the thickness increases to 150-200 microns in the apical third. Cementum also maintains the width of the periodontal ligament in response to hyper and hypofunction.

The periodontal ligament (PDL) is the connective tissue structure that surrounds the dental roots and joins the cementum with the alveolar bone proper. Figure 7. The PDL is continuous with the connective tissue of the gingiva and communicates with the marrow spaces through the vascular channels throughout bone. Radiographically, the PDL space has an hourglass shape and is narrowest at the mid-root level. The width of the PDL is approximately 0 .25mm. Figure 8. The PDL has five functions: supportive, nutritive, formative, sensory and protective. The role of state of the art tissue engineering in periodontal regenerative surgery draws from an understanding of the PDL and subjacent connective tissue. The most important elements of the PDL are the collagenous principle fibers. Figure 9. They are arranged in bundles and follow a wavy course. Terminal portions of the principle fibers, called Sharpey's Fibers, insert into cementum and bone. Figure 10. The principle fibers are arranged in the following groups based on orientation: alveolar crest fibers, horizontal fibers, oblique fibers, and apical fibers. Figure 11. Fibroblasts that form the collagen in these bundles continues to extend and remodel ligament fiber bundles. New collagen is formed and older collagen is resorbed until there is a continuous network of fibers between bone and cementum. The majority of fiber bundles are made up of type I collagen (80%) and type III collagen. Each collagen fiber is made up of smaller diameter collagen fibrils. Figure 10. Each fibril is composed of individual bundles of collagen molecules; fibroblasts secrete these molecues as procollagen alpha helices. These procollagen molecues area assembled and modified extracellularly into staggered linear pattern bundles which have the characteristic cross-banding pattern seen in the collagen fibril. Other fibers are present: elastic, oxytalan and indifferent. The cellular elements of the PDL are fibroblasts, cementoblasts, osteoblasts, osteoclasts and eptithelial cells called "epithelial rests of Malassez". Figure 12.The blood supply of the PDL is from the inferior and superior alveolar arteries and reaches the PDL via apical vessels, penetrating vessels from the alveolar bone and anasomosing vessels from the gingiva. The blood supply is critical to the success of regenerative procedures.

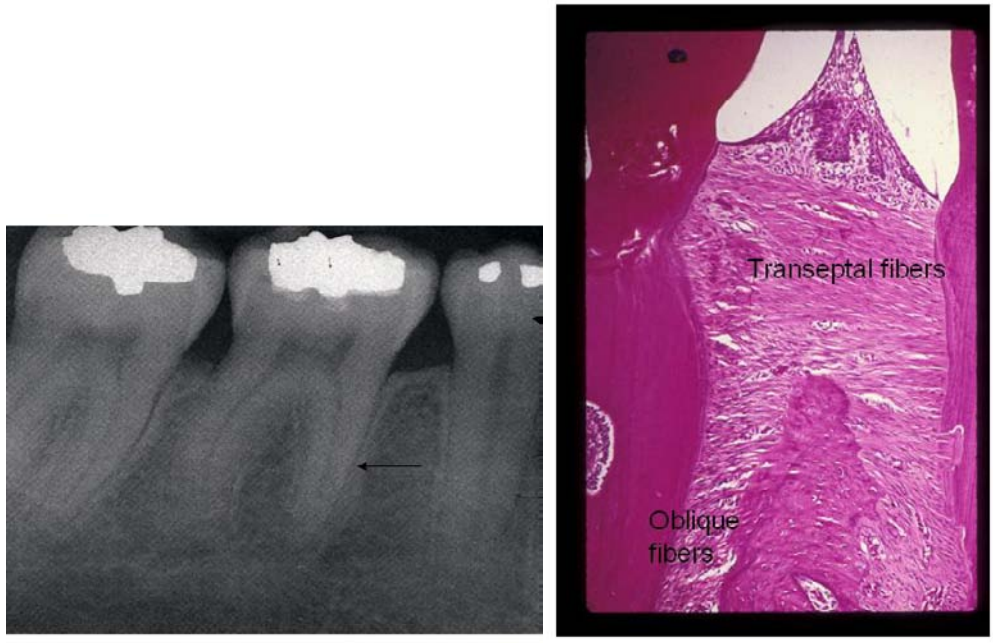


Figure 8.

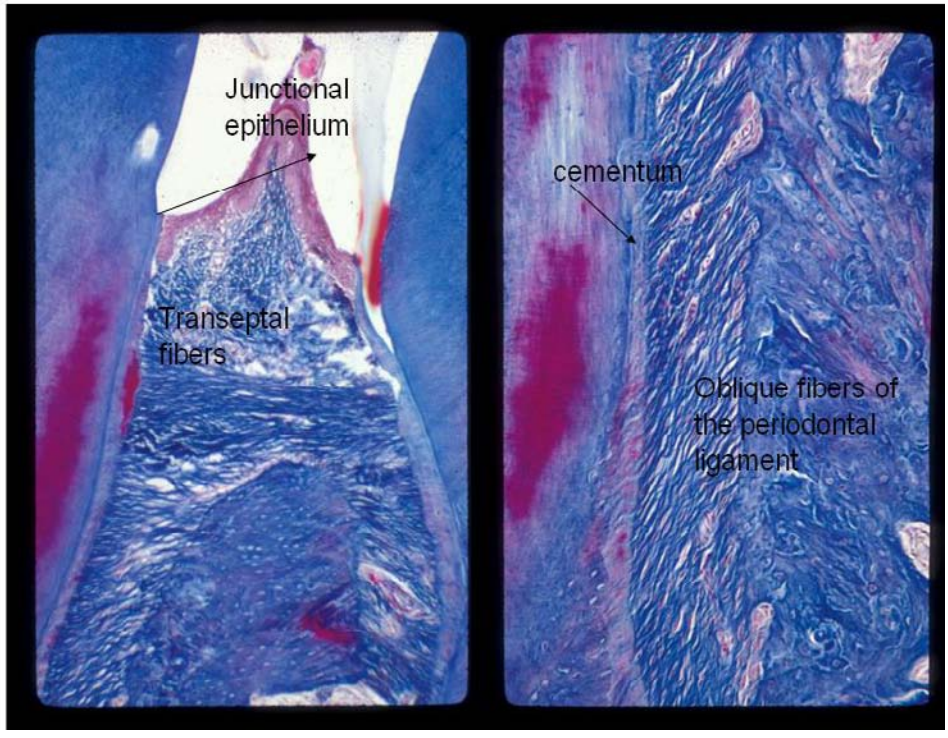


Figure 9.

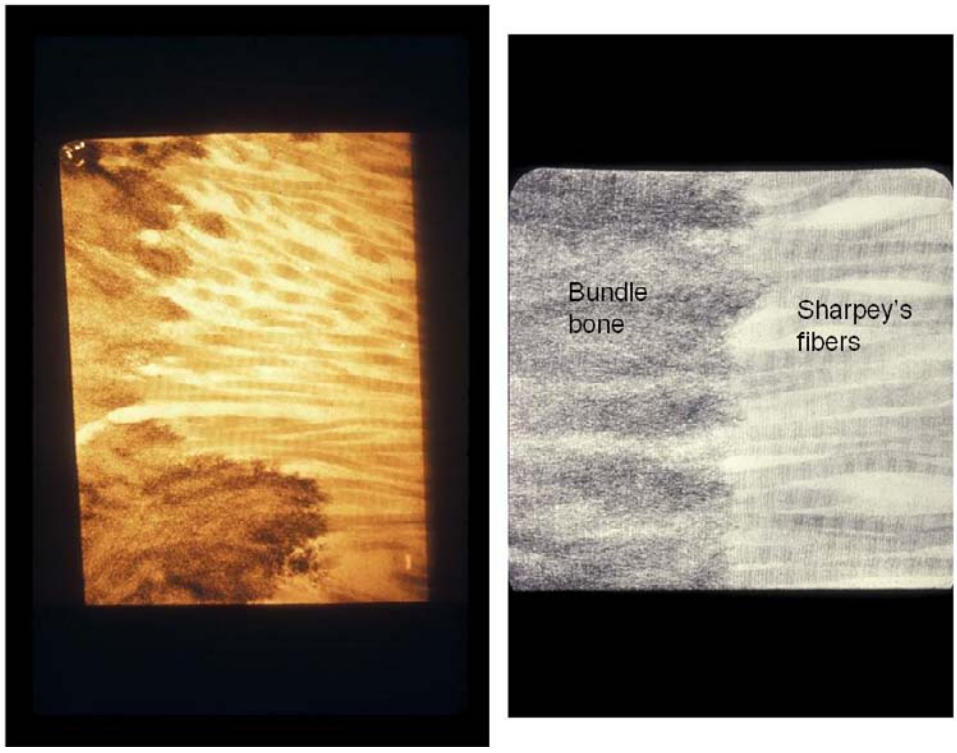


Figure 10.

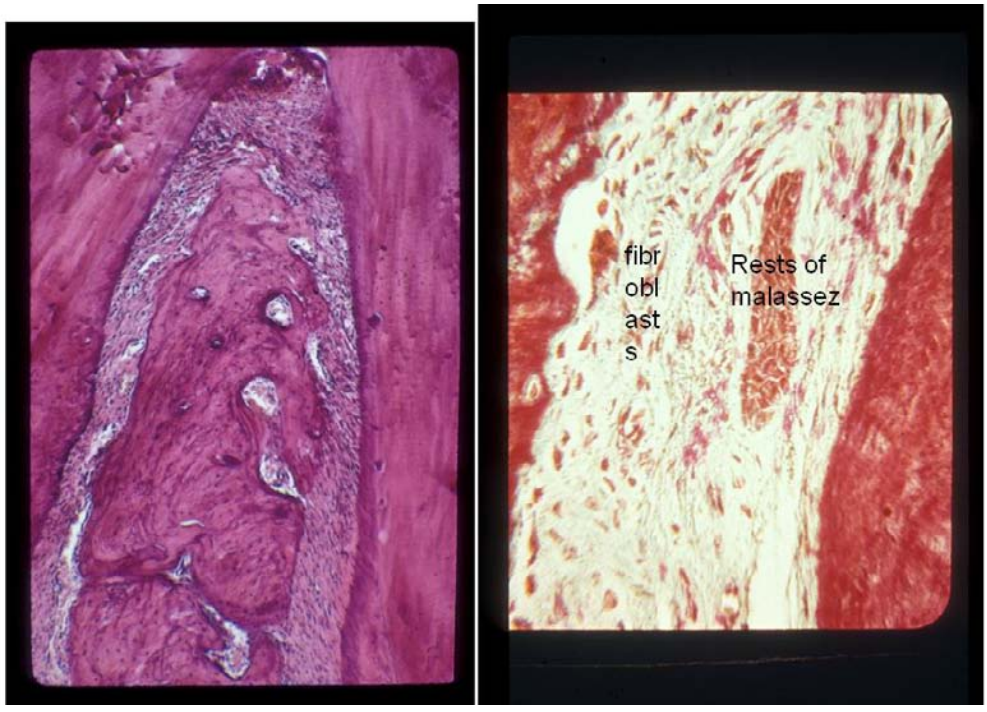


Figure 11.Figure 12.

The alveolar process is the bone that forms and supports the teeth. It consists of alveolar bone proper (formerly known as cribriform plate), made up of compact bone of the inner socket wall, and supporting alveolar bone, made up of cancellous trabeculae and the facial and lingual plates of compact bone. Bone adjacent to the PDL is called bundle bone because it contains Sharpey's Fibers. These regions of bone are visible on a dental radiograph. Figure 8. Radiographically, the alveolar bone that lines the bony socket and alveolar crest appears to be a dense line called the lamina dura. The appearance of radiodensity may be due to increased bone density in this area, or it may be the result of superimposition of the bony curvatures of the boney socket. Lack of radiographic lamina dura can be observed in periodontal diseases involving alveolar bone loss. Bone morphology has become relevant in modern periodontics because of the importance of bone contour for esthetic outcomes as well as treatment planning for dental implant and reconstructive cases. The bone contour normally conforms to the root prominence, with intervening vertical depressions that taper towards the margin. Because the height and thickness of the facial and lingual boney plates are affected by tooth alignment and angulation of the root to the bone, the morphology of bone is closely linked to esthetic presentation. Bone fenestrations are round or oval defects in the cortical plate over the root surface. Bone dehiscences occur when a lack of alveolar bone over a root extends to the alveolar crest.

References

Tarnow DP, Magner AW, Fletcher P.J Periodontol. 1992 Dec;63(12):995-6.*The effect of the distance from the contact point to the crest of bone*

Pathogenesis of Gingival Diseases

There are many types of gingival diseases. The most common is gingivitis induced by bacterial plaque biofilm. Where biofilm plays a central role in initiating disease, local and systemic factors related to the host modify the condition. (Marshall) In a population enjoying a longer life expectancy, often surviving with chronic diseases controlled with medications and therapy, the prevalence of these modifying conditions impacts every dental practice. In the presence of host related systemic risk factors which may be disease, medication, habit –or any other circumstance which affects inflammatory proclivity, wound healing potential, collagen synthesis the characteristics of the gingival disease, diagnosis, prognosis and treatment planning are affected.

Marshall RI, Bartold PM.Medication induced gingival overgrowth.*Oral Disease* 4:130-151, 1998.

Gingivitis

Gingivitis is an inflammation of the gingiva**.** Because minor inflammation waxes and wanes for long periods of time, there is no histologic dividing line between health and transient gingivitis. Clinically healthy gingiva often presents with accumulation of few inflammatory cells at times. Through a convention developed using animal studies, the description of the development of gingivitis to periodontiitis is as follows: the initial lesion, the early lesion, the established lesion and the advanced lesion (Moskow).

Plaque induced gingivitis clinically presents with the cardinal signs of inflammation: redness, swelling and bleeding upon probing. Histologically, the gingival tissue response to bacterial plaque biofilm insult begins in the sulcus. Inflammation is the hallmark of gingivitis and is accompanied with the formation of a pocket. If gingivitis progresses, loss of junctional epithelial attachment will occur. Figure13. The junctional epithelium widens and proliferates to the underlying connective tissue. In gingivitis, there is no apical migration of the junctional epithelium. Neutrophil rich inflammatory cells migrate in through the junctional epithelium into the sulcus. Neutrophils are "being called" to the sulcus for their phagocytic properties as a response to the bacterial plaque biofilm. (Seymour)

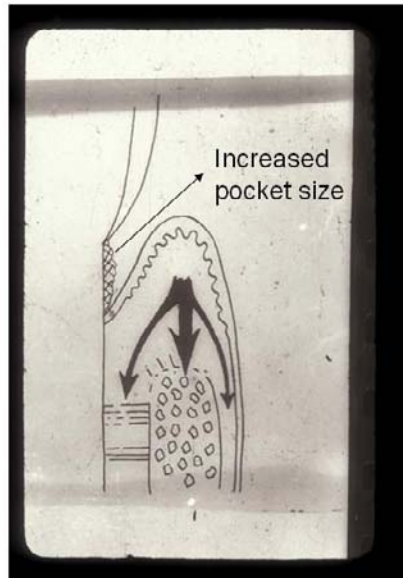


Figure 13.

Connective tissue is also exhibits histologic signs of acute and chronic inflammation. Neutrophils, mast cells, macrophages, lymphocytes and plasma cells are present. In gingivitis lesions, the inflammatory infiltrate is dominated by neutrophils and T-lymphocytes. Figure 14.

In the presence of bacterial plaque induced gingival inflammation, medications, such as calcium channel blockers and anti-convulsantscan induce gingival enlargement. Approximately one third of patients using these medications actually develop with the condition. (Philstrom) Meticulous plaque control can reduce this side effect. Hypersensitivity lesions and mucocutaneous disorders occasionally present without specific microbial

association. (Holmstrop) Individuals with poor metabolic control, as is the condition in uncontrolled diabetics,present with greater inflammation than those individuals with better metabolic control.Antibacterial host defences such as neutrophil chemotaxis and phagocytosis are considered responsible.

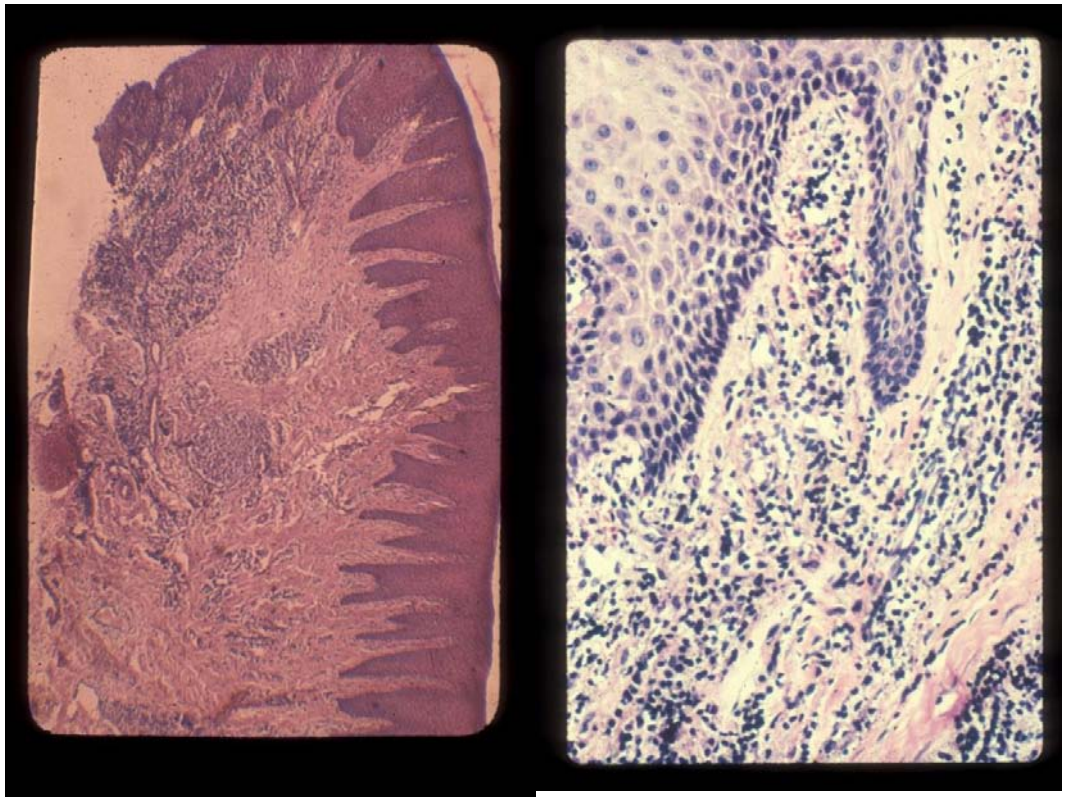


Figure 14 Low and high power magnification.

In nonplaque induced gingival disease, specific bacterial, viral, or fungal infections are responsible for inflammation. Specific bacteria such as Neisseria gonorrhea, Treponema pallidum , and specific viruses such as varicella zoster, papillomavirus, and herpes simplex type 1 and 2 and candidosis and histoplasmosis in otherwise susceptible individuals are shown to affect inflammation. (Siegel) Gingival lesions which resemble plaque-induced gingivitis occur in mucocutaneous disorders such as lichen planus, pemphigoid, pemphigus, Stevens-Johnson Syndrome, lupus (Tietmann) and psoriasis.

References

Moskow BS, Polson AM.1991.Histologic studies on the extension of inflammatory infiltrate in human periodontitis.*J Clin Periodontol* 18:375.

Seymour GJ, Powell RN, Cole KL et al.1983Experimental gingivitis in humans.A histochemical and immunological characetization of lymphoid cell populations.*J Periodont Res* .18:375.

PhilstromBL, Carlson JF, Smith QT, Preventionof phenytoin associated gingival enlargement-A 15 month longitudinal study.*J Periodontol* 1994 51:311-17.

HolmstropP. 1999 Non-plaque induced gingival lesions.Ann Periodontol 4:20-9.

Siegel MA.Syphillis and gonorrhea.*Dent Clin N am* 40:369-83.1988.

Tietmann, C., BISSADA, N.F.Aggressive Periodontitis in a Patient with Chronic Cutaneous Lupus Erythematosus: A Case Report. Quintessence Int.*Quintessence Int*.36(5):41-48.

Treatment of Plaque Associated Gingivitis

The therapeutic goal when treating plaque associated gingivitis is to establish gingival health by removing the primary etiologic agents such as plaque, calculus and other plaque-retentive factors. The host related risk factors for disease progression should be taken into consideration when planning treatment as these factors may modify response to treatment, and prognosis. A thorough evaluation of therapeutic outcomes is necessary after treatment and repeatedly at maintenance visits in light of risk profile.

Periodontitis

The hallmark of periodontitis is the irreversible (until relatively recent advent of regenerative surgery) loss of periodontal attachment apparatus structures. As is characteristic of chronic diseases, periodontitis can progress slowly or in short spurts of “activation” which ebb and flow over long periods of time periods of disease exacerbation and severity may be associated with local factors, modification with systemic disease, habits such as smoking or with poor response to stress. (Lindhe)

When pocketing associated with gingivitis progresses, blood vessels adjacent to the junctional epithelium become enlarged and more permeable. Neutrophils migrate out of the permeable vessels and into the pocket area. Collagen around the blood vessels is lost. Lymphocytes and macrophages accumulate apical to the junctional epithelium. Fibroblasts show pathologic changes, and collagen is lost apical to the junctional epithelium. In chronic periodontitis, there is apical migration of junctional epithelium. As a result, epithelial cells become attached to the root surface cementum and the pocket wall is covered by pocket epithelium. The underlying connective tissue exhibits more B-lymphocytes which subsequently transform into plasma cells. Pocket formation results in a space, or sort of “pot-hole” which can retain bacteria. Figure 15. The chemical conditions in this pocket environment, favor proliferation and colonization of periodonto-pathogenic bacteria. The products of these pathogens further challenge the host immune defense, as underlying connective tissue changes found in gingivitis spread apically along the root surface. When the inflammatory process encounters the dentogingival fibers and periodontal ligament fibers, attachment apparatus is lost through the action of collagenases from PMNs and fibroblasts. When inflammatory process reaches the alveolar bone crest, osteoclastic bone resorption

occurs. Figure 16, 17 The inflammatory cells activate cytokines such as IL-1, TNFalpha and IL-6. Bone destruction occurs though osteoclastic action which is triggered by cytokines and inflammatory mediators such as IL-1Beta and PGE2. (Seymour)

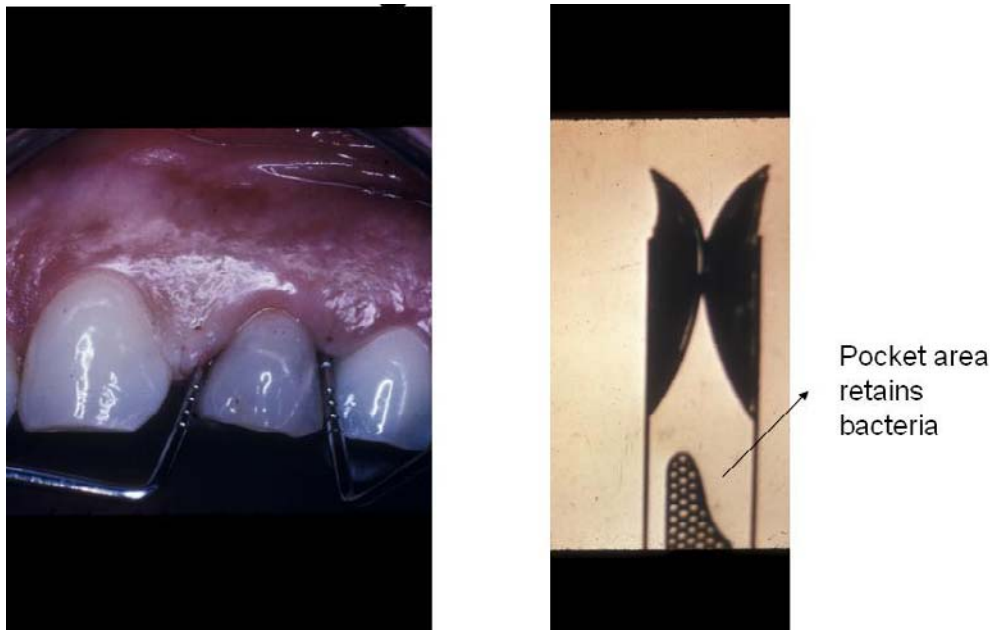


Figure 15.

Figure 16.

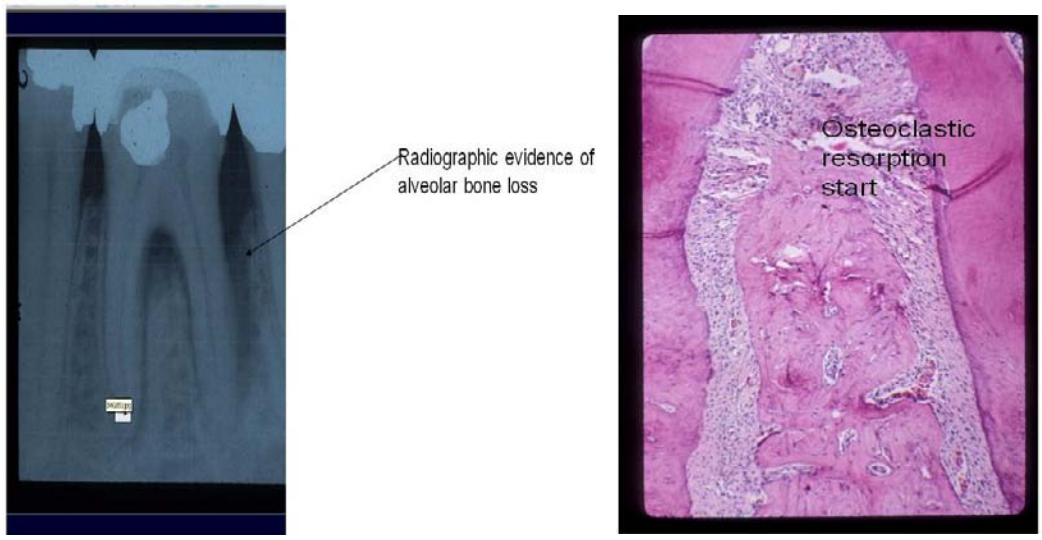


Figure 17.

References

Lindhe, Ranney, Lamster.Concensus report: chronic periodontitis, *Ann Periodontolol* 4:38, 1999

Seymour GJ, Powell RN, Cole KL, Aithen JF, Brooks D, Beckman I.Experimental gingivitis in humans.A histochemical and immunological characterization of the lymphoid cell subpopulation.*J periodont Res* 198318:375.

Chronic Periodontitis

Chronic periodontitis is a common, multifactorial, plaque-induced disease. It has been considered an infectious disease and an inflammatory disease. It is the most common form of periodontal attachment destruction in adults, but is also seen in primary and permanent dentition in children. Clinical features include cardinal signs of inflammation: edema, gingival bleeding on probing, and suppuration. Figure 18,19. The current system of classification was set forth by Armitage in 1999 and includes chronic periodontitis. It uses clinical attachment loss as the "measuring stick" to determine, mild, moderate or severe involvement. Depending on the number of sites in the mouth exhibiting loss of attachement, the diease is classified as localized (less than 30% of sites), or generalized (more than 30% of sites).

Chronic periodontitis is triggered by a host immune response to bacterial plaque biofilm, but this response is modulated by a number of host associated, local and environmental factors. Because of the multifactorial nature of chronic periodontitis, disease progression is primarily prevented by disruption of the bacterial plaque biofilms which triggered it in the first place. Disease progression and prognosis vary based on host specific risks, but the

overall therapeutic goal is to restore function and esthetics while preventing continued attachment loss.

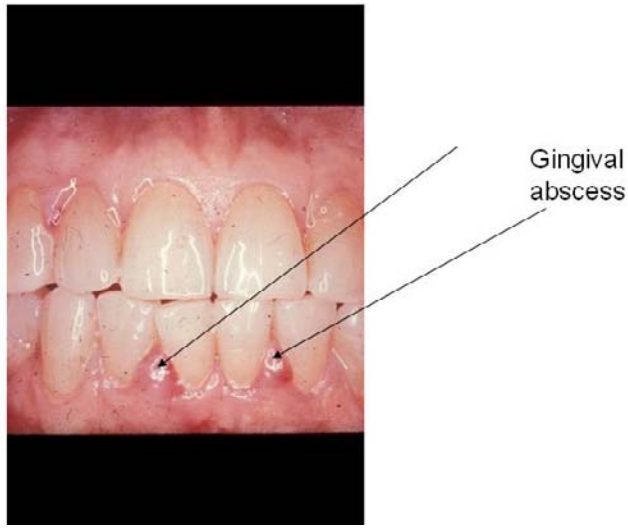


Figure 18.

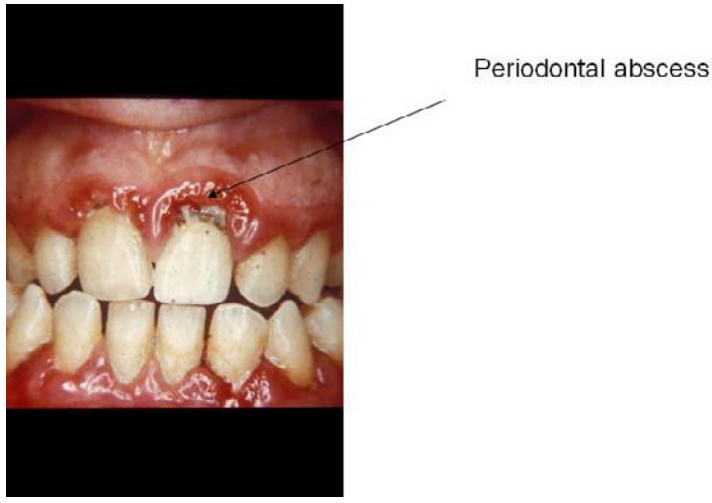


Figure 19.

Aggressive Periodontitis

Aggressive periodontitis includes distinct types of disease which all have a rapid rate of disease progression. It occurs in localized and generalized forms, both having different microbial etiology (Lang). The amount of microbial deposits are inconsistent with the severity of tissue destruction. Aggressive periodontitis can occur at any age, and tends to occur in patients who appear otherwise healthy. (Cogen) Disease progression is self-limiting.

Localized aggressive periodontitis presents with damage localized to fist molars and incisors is usually identified in individuals around puberty.*Actinobacillus actinomycetmcomitans* and robust serum antibody response are associated with this presentation. Generalized aggressive periodontitis presents with damage to interproximal sites in at least 3 permanent teeth other than first molars and incisors. Episodic attachment destruction occurs and is associated with *Actinobacillus actinomycetmcomitans and Porphyromonas gingivalis* and a poor serum antibody response.(Christersson) In both disease types, neutrophil dysfunction is noted. (Cianciola)

The goals of therapy are to stop disease progression by disrupting periopathogens. Because of the distinct disease types which make up aggressive periodontitis, multiple strategies are useful. Treatment may be similar to that of chronic periodontitis: scaling and root planing with microbial indentification and antibiotic sensitivity testing, adjunctive antimicrobial therapy, control of local plaque-retentive factors, occlusal therapy, periodontal therapy and frequent periodontal maintenance stressing oral hygiene instructions. (Mandel, Novak) Additionally, general medical evaluation, and physician consultation to identify underlying systemic etiology may be indicated.

References

Lang N Bartold *Ann Periodontol* 4:53, 1999

Cogen RB, Wright JT, Tate AL. Destructive periodontal disease in healthy children. *J Periodontol* 1992;63: 761-765.

Christersson LA. Actinobacillus actinomycetemcomitans and localized juvenile periodontitis. Clinical, microbiologic and histologic studies.*Swed Dent J* (Suppl. 90) 1993;90:1-46.

Cianciola LJ, Genco RJ, Patters MR, McKenna J, van Oss CJ. *Defective polymorphonuclear leukocyte function in hu Nature* 1977;265: 445-447.

Mandell RL, Socransky SS. Microbiological and clinical effects of surgery plus doxycycline on juvenile periodontitis. *J Periodontol* 1988;59:373-379.

Novak MJ, Polson AM, Adair SM. Tetracycline therapy in patients with early juvenile periodontitis. *J Periodontol* 1988;59:366-372.

Acute Conditions

Pain, discomfort and infection characterize acute conditions of the periodontium. Acute periodontal infections include gingival and periodontal abscess, necrotizing periodontal diseases, herpetic gingivostomatitis, periocoronitis, and combined perio-dendodontic lesions. Abscess is a localized purulent infection. Gingival abscess involves the marginal gingival or interdental papilla. Figure 18. Periodontal abscess involves the tissues adjacent to the periodontal pocket; periodontal abscess may lead to the destruction of the PDL and alveolar bone. Figure 19. The therapeutic goal is to eliminate acute signs and symptoms. Treatments may include establishing a pathway for drainage, irrigation, limited occlusal adjustment and use of antimicrobials and medications for pain management.

Necrotizing ulcerative gingivitis (NUG) may occur limited to the gingival or progress to include attachment loss. In this case it is called necrotizing ulcerative periodontitis (NUP). Figure 20. Classic presentation of these diseases includes necrosis and ulceration of the interdental papillae at the tip and gingival margin which create "punched out" gingival appearance. Figure 21. Gingiva is painful and bright red. Malodor and gingival bleeding are present. This condition is associated with immune compromised conditions such as HIV/AIDS. The goal of therapy is to promptly eliminate acute signs and symptoms. Irrigation, debridement, pain control, systemic antibiotics and local antimicrobials and and oral hygiene instructions are all treatment considerations. Additionally, patient counseling on smoking cessation and nutrition along with comprehensive periodontal evaluation should follow after the acute condition is resolved.

Figure 20

Herpetic gingivostomatitis is a viral infection which presents clinicially with generalized pain in the oral mucous membranes and gingival, gingival inflammation, vesiculation and ulceration, and lymphadenopathy, fever and malaise. Therapy, including debridement and pain control with systemic medication and topical anesthetic rinse, is aimed at pain relief. The goal of therapy is to facilitate hydration, nutrition, and oral hygiene.

Pericoronitis is a local purulent infection associated with a gingival flap around the crown of a tooth which is often partially erupted. Pericorontis presents as localized edematous, painful lesions which may have purulent exudate, lumphadenopathy, fever, and malaise. In cases of severe swelling, trismus may also occur. The goal of treatment is to prevent further swelling and spread of infection. Once infection is controlled with antibiotic therapy, debridement, irrigation and tissue recontouring or tooth extraction follows.

Figure 21

When pulpal inflammatory infection communicates with the oral cavity through periodontal attachment apparatus, either PDL or alveolar bone, there is a combined perio-endodontic lesion. Often well circumscribed, and sometimes associated with a fistulous tract, these lesions also are associated with tooth fracture. Clinical presentation includes swelling, sensitivity and tenderness, similar to acute lesions noted above. As with other acute lesions, therapy for perio-endodontic lesions aims to eliminate pain and swelling using similar methods. Surgical access for debridement and localizing tooth fracture may be useful along with comprehensive endodontic and periodontal examination.

Examination

Examination is an initial step in disease identification and is repeated frequently, such as at re-evaluation of initial therapy, after non-surgical, and surgical therapy to identify disease progression. Since the presence of a periodontal pocket suggests previous attachment loss, measuring the periodontal pocket depth and assessing for presence of inflammation signs is the start point of examination and diagnosis. Probing depth is measured on six sites per tooth: mesiofacial, facial, distofacial, mesiolingual, lingual, and distolingual. Periodontal probe depth measures the depth of the pocket plus the inconsistent amount of connective tissue penetration which depends on the presence or absence of inflammation and resistance offered by the epithelium. Figure 22 Additionally a number of other variables affect probe depth measurement: probing force, angulation, and location. Various types of probes with varying diameters, and varying markings are available. Additionally, force controlled probes are available to examiner error. (Jeffcoat) Histologic sections show that the tip of the probe is not

likely to stop at the coronal extent of junctional epithelium, but rather penetrate apical to the coronal level of the attachment and overestimate the clinical probe depth. Such an impingement is more likely in the presence of inflammation or when viewed from an angle. (Saglie). Caution must be used in interpreting probe depth during an examination as the tip of the probe is meant to stop at the level of junctional epithelium and not at level of bone crest. When transgingival probing measurements are compared to measurements taken when a mucoperiosteal flap is reflected, transgingival probing was identical to surgical measurement 60% of the time. In the presence of intrabony defects, high correlation between transgingival probing is reported. (Ursell)

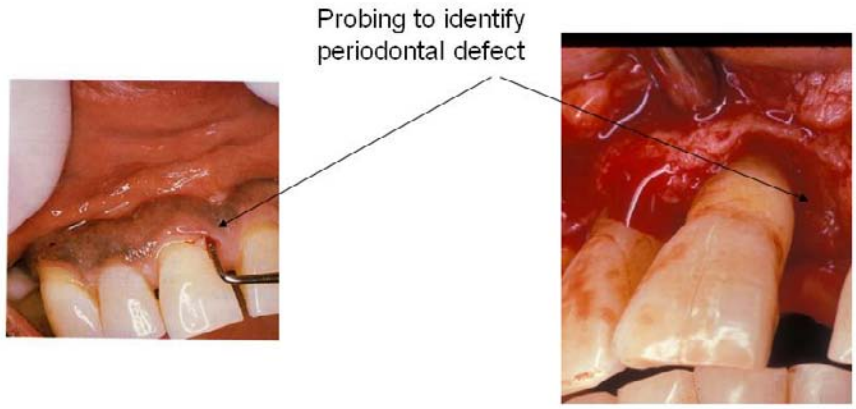


Figure 22.

Probe depth clinically measures the distance from the gingival margin to the base of the sulcus or pocket. This is the distance, histologically, to the junctional epithelium, Clinical attachment loss is calculated as the distance from the cementoenamel junction, a fixed landmark, to base of the sulcus or pocket. CAL is more complete and encompassing measure, since it incorporates the location of the gingival margin, and the presence of recession or hyperplasia. Therefore CAL measurements are a more valid outcome measure.

Tooth mobility is measured during examination. Miller Mobility Index (Miller) is commonly used clinical index for measuring mobility. Mobility is detected by using two metal (that is to say no deformable) instruments on either side of the tooth and applying force.. Physiologic mobility is movement that occurs under healthy conditions. Pathologic mobility occurs when the PDL is damaged by the disease processes which injure collagen fibers and result in alveolar bone loss. Pathologic mobility may be 10 times greater than physiologic mobility.

References

Ursell J. Relationships between alveolar bone levels measured at surgery, estimated by transgingival probing and clinical attachment level measurements. *J Clin Periodontol* 1989;16:81-86.

Saglie R, Johansen JR, Flotra L. *The zone of completely and partially destructed periodontal*

Jeffcoat MK, Jeffcoat RL, Jens SC, et al. A new periodontal probe with automated cemento-enamel junction detection.*J Clin Periodontol* 1986; 13:276-280.

Position paper *J Periodontol* 2003;74:1237-1247

Armitage GC. Manual periodontalprobing in supportiveperiodontal treatment.*Periodontol* 2000 1996;12:33-39.)

Examining Mucogingival Conditions

With the increased interest in esthetic outcomes, and greater predictablitly of soft tissue and plastic surgical procedures in periodontics, the examination of mucogingival condition has gained importance.Gingival recession is root surface exposure by an apical shift in gingival position. (Figure 23) The appearant position is the level of the crest of the gingival margin, but since the actual postion is the level of the epithelial attachment, this actually determines the severity of recession.There fore the distance from the CEJ to the gingival margin determine the appearant position of gingival recession, but probe depth measurement is needed to determine the actual potion of the epithelial attachment.A mucogingival defect occurs when the base of the pocket is at or beyond the mucogingival junction.Because recession is only clinically observable in some cases, but is often hidden by inflamed gingiva, both these measurements are important.Additionally, after initial therapy, such as scaling and root planing or prophy, when gingival inflammation subsides, re-examination is needed to identify recession. Recorded baseline and re-examination data should be compared in order to determine a need for treatment.

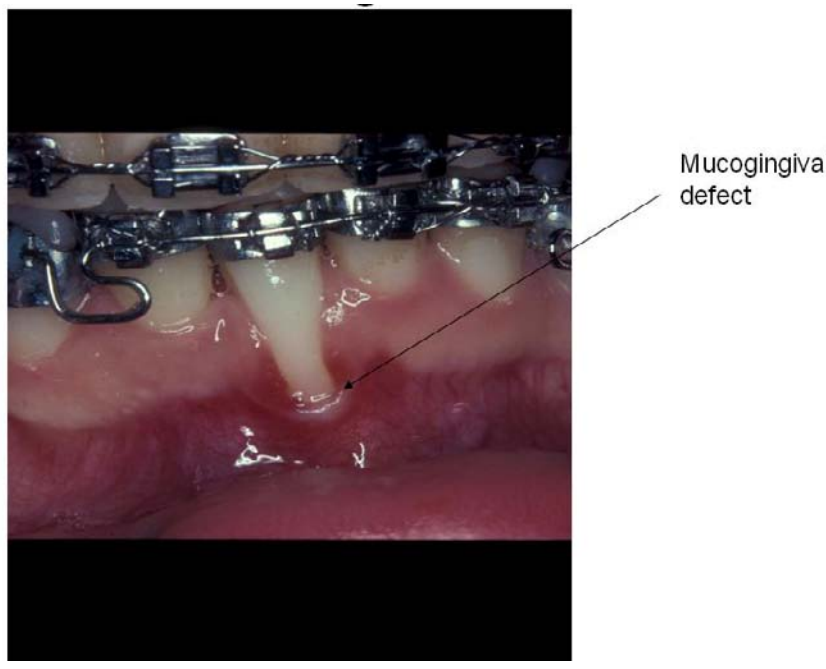


Figure 23.

Since developmentally some areas of the mouth, such as mandibular premolar region, present with less attached gingival than other areas, it is intuitive that some areas are more prone to mucogingival defects. Additionally, anatomic variations such as tooth postion, frenum insertion, vestibular depth and ridge anatomy contribute the presentation. Whether or not to treat mucogingival defects is studied in both natural teeth and dental implants.In inflamed sites, keratinized tissue offers more protection than alveolar mucosa.Therefore, cautious and continuous assessment of gingival inflammation in areas with limited zone of keratinized tissue is needed.In natural teeth, it is concluded that a width of keratinized tissue of 2mm where at least 1 mm is attached is adequate to maintain gingival health.(Lang).When natural teeth are restored, higher gingival inflammation was noted on teeth with less than two mm of keratinized tissue (Stetler).Mucogingival problems may progress with age, although zone of attached gingival also increases with age.

The need for keratinized gingival in dental implant outcomes is controversial. The limited (less than 2mm) zone of keratinized tissue especially in posterior sites is associated with higher plaque accumulation and gingival inflammation. It is also identified that in two stage dental implants, failure can occur as a result of progressive soft tissue inflammation beginning at the gingival crevice. (Kirsch A)4 year evaluation of immediate loaded implants placed in fresh extraction sockets shows that although there is no difference in bone level maintenance, the width of keratinized tissue is significantly associated with less gingival inflammation, plaque accumulation and gingival recession. (Bouri) Controversy and continuously emerging evidence in the effects of keratinized tissue zone on dental implant outcomes places a heavy responsibility on the clinician to continually examine and re-examine the parameters associated with attachment, inflammation and bone height.

References

Stetler KJ, Bissada NF. Significance of the width of keratinized gingival on the periodontal status of teeth with submarginal restorations. *J Periodontol* 1987;58:696~700.

Maynard JG, Wilson R. Diagnosis and management of mucogingival problems in children. *Dent Clin N Am* 1980;24:683-703.

Lang N, Loe H. The relationship between the width of keratinized gingival and gingival *health. J Periodontol* 1972;43:623-627.

Kirsch A, Mentag PJ. The IMZ endosseous two phase implant system: A complete oral rehabilitation treatment concept. *J Oral Implantol* 1986;12:576-589.

Bouri, A., Bissada, N.F., Ficara, F., Fadoul, F., Al-Zahrani, M. Width of Keratinized Gingiva and the Health Status of the Supporting Tissues Around Dental Implants. *Int. J. of Oral & Maxillofacial Implants*.23(2): 323-26.

Use of Indexes for Documenting Examination Findings

The common index systems allows clinicians to document subjective findings related to periodontal parameters.Documenting inflammatory status along with probe depth, gingival

recession, zone of keratinized tissue, and clinical attachment level, is a challenge because inflammatory status cannot be measured with a measurement device and the finding cannot be documented as a number of millimeters.Indexes are designed to facilitate this documentation.The presence of bleeding on probing is considered a predictor for disease progression.Since the early signs of inflammation, such as early and initial lesions in gingivitis noted above, are visible histologically, bleeding on probing is considered a useful indicator when these states of disease are present.Additionally, when inflammation is recurrent, bleeding on probing suggests disease activity.

Since the primary etiology of gingival inflammation is bacterial plaque biofilm, an index to evaluate oral hygiene status is useful.Tracking the percentage of sites covered with bacterial plaque biofilm is useful, butPlaque index of Silness and Loe is commonly used in epidemiological studies. (Silness)

References

Loe H, SilnessJ, Periodontal disease in pregnancy I prevalence and severity .*Acta Odontol Scand* 1963;21:532-551.

Loe H.The gingival index, the plaque index and the retention index systems.*J Periodontol* 1967;38:610-616.

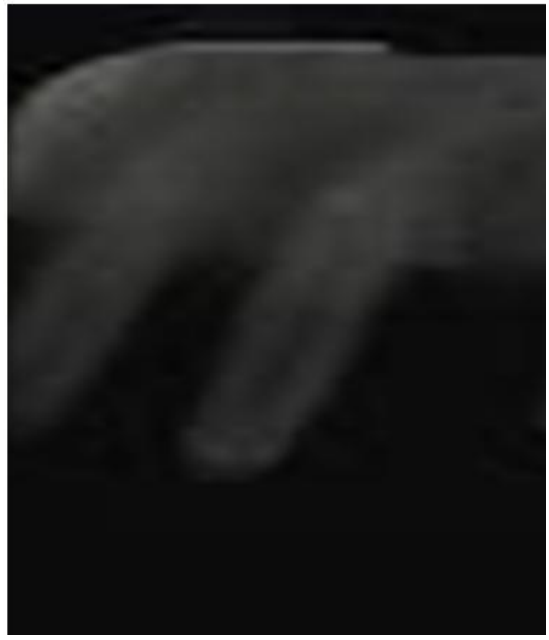
Radiographic Aids in Periodontal Examination and Diagnosis

Radiographs aid in but cannot replace complete clinical examination.Radiographs record septal bone position on the tooth when taken and viewed in one plane. They also serve as an adjunct to clinical exam in several ways including: record alveolar bone and PDL on the mesial and distal sides of a root surface, document crown to root ratio Figure 24, and in interproximal surfaces reveal metal restorative margins and calculus deposits. Figure 25. Since soft tissue is not appearant on radiographs; they are unable to show periodontal pockets or the relationship of soft and hard tissue. Additinionally radiographs cannot distinguish between treated and untreated cases. Conventional radiographs, whether digital or analog are two-dimensional in nature and often superimpose buccal and lingal aspects of the tooth. As a result they fail to show the morphology of intrabony defects, but do suggest the presence. Figure 26.

Interdental bone crests, in the absence of periodontal disease, are determined by the positions of the neighboring CEJ. In the presence of disease, pathologic processes determine the alteration in the interdental septa. Crown shape and size, tooth postion, and state of eruption or migration can determine interseptal shape. Lamina dura, the radiographic analog of the cribriform plate of alveolar bone is generally absent during active disease. Although lamina dura has been created artificially on radiographs (Manson), it is generally accepted that the integrity of the crestal lamina dura is an indicator of periodontal health. (Greenstein)

Modern periodontics has been empowered through new imaging technologies. Digital radiography has many uses for periodontic treatment. Since digital radiography produces

images in jpeg file format, the intermediate step of scanning to convert a conventional image to jpeg, is avoided. This format facilitates image transfer between clinicians, during the treatment planing, lab, and outcomes assessment stages. Additionally,subtraction radiography programs work directly on digital radiographs. Subtraction is a technique which compares the gray level of two images. Differences in bone loss or gain are useful in identifying periodontal disease progression, and post-operative outcomes. (Reddy).



Crown to root ration is greater than 1:1.

Figure 24

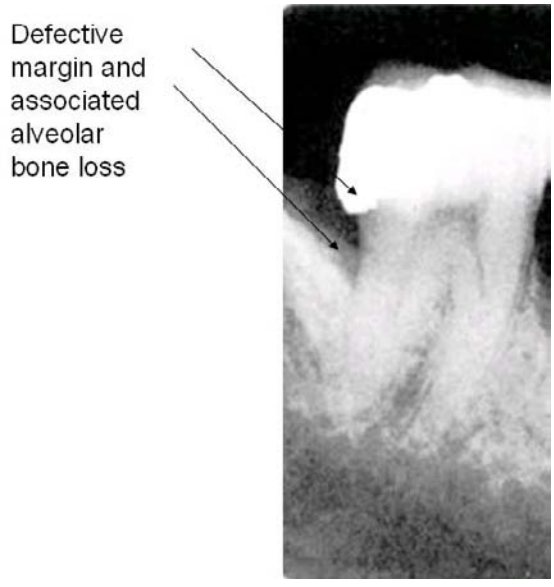


Figure 25.

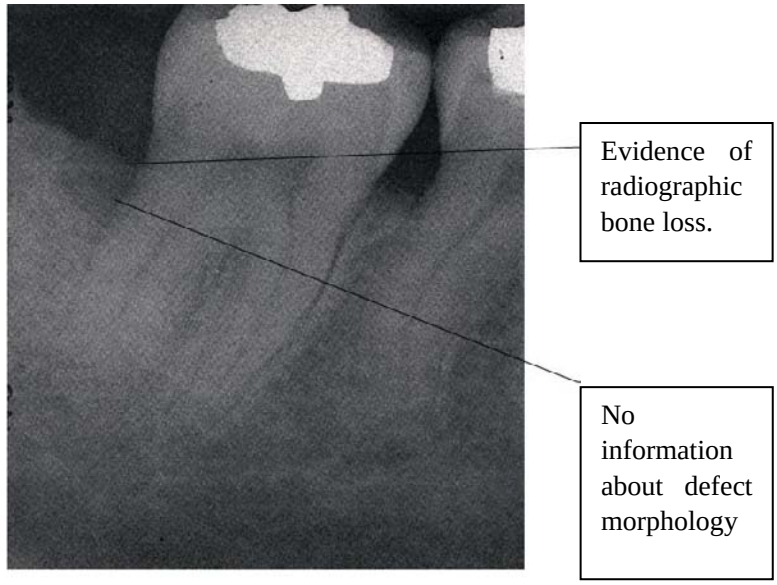


Figure 26

Three dimensional imaging, such as cat scan, also work on a digital format and will be discussed later on.

References

Reddy M. Radiographic methods in the evaluation of periodontal therapy.*J Periodontol* 1992;63:1078-1084.

Greenstein G, Poison A, Iker H, Meitner S. Associations between crestal lamina dura and periodontal status. *J Periodontol* 1981;52:362-366

Manson JD. Lamina dura.*Oral Surg Oral Med Oral Pathol* 1963;16:432- 438.

Assessing Occlusion

Ericson and Lindhe concluded that the permanently increased mobility had no influence on the development of periodontitis. Perrier and Polson induced experimental periodontitis in an animal model and imposed trauma in the presence of good plaque control and showed that trauma in a reduced periodontium cased no additional attachment or alveolar bone loss if inflammation was controlled with effective plaque control.

References

Ericsson I, Lindhe J. Lack of significance of increased tooth mobility in experimental periodontitis. *J Periodontol* 1984;55:447-552.

Miller SC. *Textbook of Periodontia,* 3rd ed. Philadelphia: Blackston; 1950: 125.

Perrier M, Polson A. Periodontol.1982 Mar;53(3):152-7. *The effect of progressive and increasing tooth hypermobility on reduced but healthy periodontal supporting tissues.*

Etiology

Bacterial plaque biofilm is the primary etiology of periodontal diseases. A biofilm is an organized mass consisting mainly of microbes and their products along with organic, polysaccharide and protein matrix. It is co-operative bacterial environment which develops in phases.The earliest phase, the pellicle, consists of mucosal salivary proteins and adheres to tooth, dental implant, restoration, and prosthetic surfaces;initial colonizers such as A. viscosus and S. sanguis adhere to the pellicle through adhesins.Secondary coloizers such as P. intermedia, P. loescheii, C. species, F. nucleatum and P. gingivalis coaggrigate and form as a symbiotic community.As the biofilm colonizers mature from gram positive cocci to gram negative bacillus and spirochetes or flagellated bacteria, the biofilm environment trasitions from an early aerobic to highly oxygen deprived environment. Colony growth depends on temperature, pH, re-ox potential, available nutrients and favors those bacteria that can successfully compete. In Chronic periodontitis, the group of bacteria common to chronic periodontitis lesions is known as “red complex” bacteria and includes T. denticola, P. gingivalis and T. forsynthesis. (Socransky).In Aggressive periodontitis,A. actinomycetemcomitans, C. Sputigena and Mycoplasma and spirochetes dominate the biofilm.(Saglie Zambon) .

References

Saglie R, Newman MG, Carranza, FA Jr, Pattison GL. Bacterial invasion of gingiva in advanced periodontitis in humans. *J Periodontol* 1982A; 53:217-222.

Saglie FR, Carranza FA Jr, Newman MG, et al. Identification of tissue invading bacteria in juvenile periodontitis.*J Periodont Res* 1982B;17: 452^154.

Saglie FR, Carranza FA Jr, Newman MG. The presence of bacteria within the oral epithelium in periodontal disease. I. A scanning and electron microscope study. *J Periodontol* 1985;56:618-624.

Zambon J. *Actinobacillus actinomycetemcomitans* in human periodontal disease.*J Clin Periodontol* 1985; 12:1-20.

Zambon J. Microbiology of periodontal disease In: Genco RJ, Goldman HM, Cohen DW, eds. *Contemporary Periodontics.* St. Louis: CV Mosby; 1990;147-160.

Bacterial plaque biofilm can be super or subgingival.If undisturbed the sticky removable layer mineralizes into hard, flint-like calculus.Calculus is an irritative factor, and hence is a

contributing etiology for periodontal diseases.Figure 27.Since the surface of calculus is always coated with un-mineralized plaque, the primary etiologic agent is always present**.**

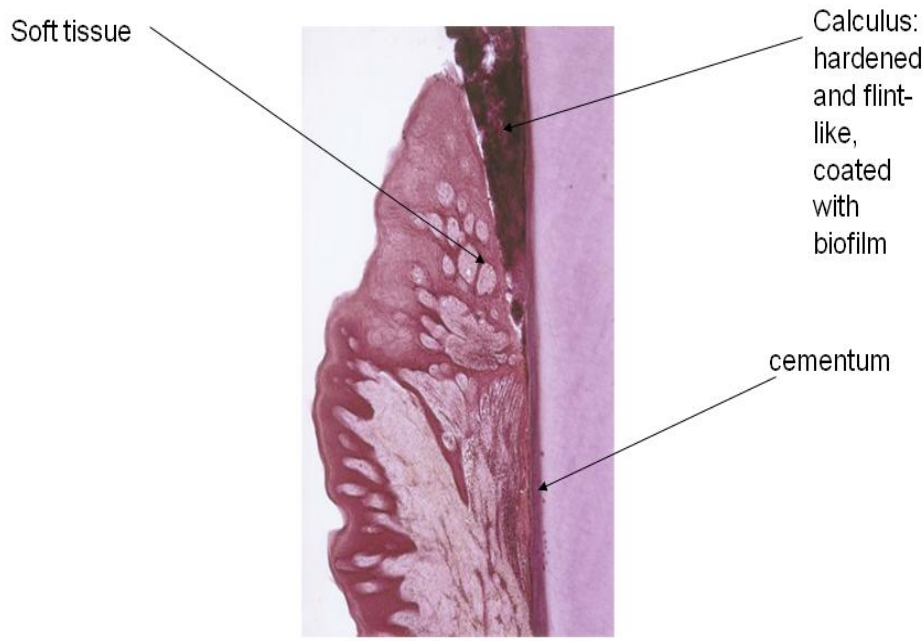


Figure 27.

The host's immune response to the primary etiology, bacterial plaque is in the form of inflammatory cascade.This inflammatory cascade generatesmatrixmetalloproteinases, collagenases and cytokines with tissue destructive effects.When the host response is hyper attenuated, tissue destruction is increased.In this way, a hyper-inflammatory phenotype can contribute to periodontal disease etiology.

Similary, an immuno-compromised condition can also contribute to periodontal disease.In conditions such as agranucytosis, cyclic neutropenia Chediak-Higashi syndrome and lazy leukocyte syndrome in which the PMN response is deficient, a minor change in the presence of bacterial plaque biofilm may lead to disease progression which is greater than what would be expected in an individual with normal immune system.Earlyperiodontal lesions are dominated by T-lymphocytes and Advancedlesions are dominatedby B-lymphocyte and plasma cells.

Local Anatomy, such as poor tooth alignment,contributes to periodontal disease.In crowded areas, oral hygiene may be compromised and predispose the area to inflammatory reaction. Similarly, restorative margins and areas which are difficult to reach with a toothbrush or floss may be apt to accumulate bacterial plaque biofilm, the primary etiology of periodontitis. Figure 28 A correlation between interproximal distance and intraboney pockets has been shown (tal); and upon examination of 29 human skulls, when the inter-root distance was less than 0.3mm, alveolar bone was not present (Heins and Wieder) . Conversely,if contacts are open due migration associated with missing teeth, open spaces, food impaction contributes to plaque-induced inflammation and trauma from forceful wedging.Additionally, uneven marginal ridges (Philstrom), plunger cusps, overbite and defective restorations can

contribute to poor interdental contacts.(Carranza). Developmental anatomic anomaly such as palato-gingival grooves and cervical enamel projections contribute to periodontal disease as well.

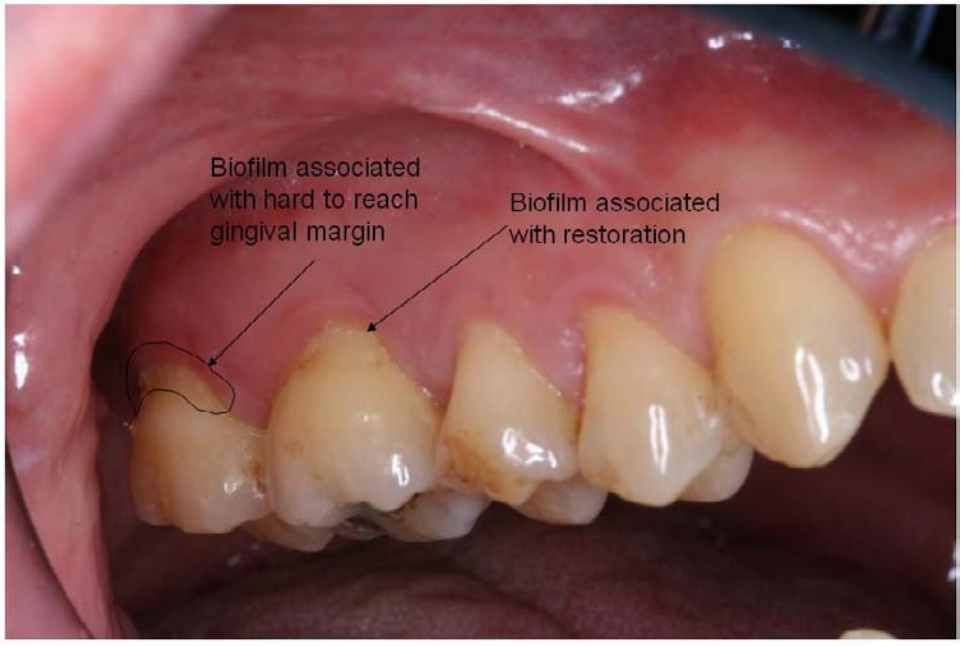


Figure 28.

References

Tal H. Relationship between the interproximal distance of roots and the prevalence of intrabony pockets.*J Periodontol* 1984;55:604-607.

Pihlstrom BL, Anderson KA, Aeppli D, Schaffer EM. Association between signs of trauma from occlusion and periodontitis.*J Periodontol*1986;57:l-6.

Carranza FA Jr. *Gllckman 's Clinical Periodontology,* 7th ed. Philadelphia: WB Saunders; 1990.

Heins P, Wieder S. A *histologic study of the width and nature of interradicular spaces in*

Role of Risk Assessment in Periodontics

The AmericanAcademy of Periodontology's statement on Risk assessment defines risk assessment as the process by which qualitative or quantitiative assessments are made of the likelihood of adverse events to occur as a result of exposure to specified health hazards or by the absence of beneficial influences (AmericanAcademy of Periodontolgy). Since prevalence and incidence rates are important to developing odds ratios for periodontal conditions, epidemiologic studies often serve as important references when interpreting risk.

Recent research findings have generated thought about the concept of risk assessment as part of diagnosis, prognosis and treatment planning in chronic disease such as diabetes, cardiovascular disease and periodontitis. Risk assessment goes beyond identification of the disease and its severity; it considers the future chronic disease presentation, including disease progression and prognosis. This concept enables the clinician to take on the role of "risk manager".

This new evolution is in marked contrast to the classical role of the periodontist. In the classic paradigm, periodontal diseases are seen as the same from individual to individual, with no consideration of newly identified host-based risk factors, then all periodontal treatments follow a similar rubric. Patient outcomes are limited in outdated treatment models and are likely to conclude with "cookie-cutter" early intervention in the form of scaling and root planing. The consequence of limiting therapy to initial therapy prescribed without risk based critical decision making is that patients may have more advanced conditions by the time more sophisticated treatment is delivered. Risk assessment challenges the clinician to identify at risk patients early and to, in a proactive way, provide targeted treatments. Such an approach aims to reduce the need for more complex periodontal procedures, lack of function and comfort associated with attachment loss and tooth loss. As such, an important goal of this segment is to educate clinicians about the importance of assessing risk while patients have only mild periodontitis.Those patients may have greater treatment needs over the course of their lifetime and require greater levels of professional monitoring.

Since periodontal attachment apparatus is destroyed in part by proinflammatory cytokines, oxygen reactive species and matrix metalloproteinases brought about by host immune activity, risk factors for periodontal diseases-any characteristic, environmental or systemic, modifiable or not, which is associated with destruction of periodontal attachment-are important to understand.Assessing risk factors allows the clinician to predict future disease progression and prognosis of treatment.It allows us to focus our clinical efforts not only on the control of bacterial biofilm plaque triggers for periodontal attachment loss, but also to potentially change the host response to it.Based on recent research efforts, clinicians can even aim to reduce destructive mechanisms in some patients.

References

American Academy of Periodontolgy vol 79 vol 2 2008

Risk Factors Whichare Not Modifiable by the Patient

Age

Periodontal disease prevalence and severity are increased in older age groups. (Hugoson, Brown). Since attachment loss is cumulative over a lifetime, it is intuitive that the older the patient, the greater the attachment loss. Some studies have shown greater bacterial biofilm plaque and more severe gingivitis in elderly populations and suggest this as an age-related

effect. Elderly patients also show an increased incidence of infections.(Grossman) Immune changes may be partly responsible for differential response of elderly patients to bacterial biofilm insult. (Kay) Age-related reduction in cell-mediated immunity has been identified through a decreased response to foreign antigens. (Weksler). Additionally, older patients have presumably had a more long term exposure to antibiotic therapy. Antibiotic therapy has been shown to reduce myeloperxidase activity, which is important to the PMN bacteriocidal function. The effect of long term antibiotic therapy on periodontal disease through altered PMN function requires more study.

Americans are enjoying a longer life expectancy and more and more people are living longer with chronic diseases controlled by medications. (Baker). Because medications impact periodontal condition in different ways, the effects can have a variety of outcomes after years of chronic use.

References

Hugoson A, Jordan T.Frequency distribution of individuals aged 20-70 years according to severity of periodontal disease.*Comm Dent Oral Epidemiol*.1982;10:187-92.

Brown LJ, Loe H.Prevalence, extent, severity and progression of periodontal disease.*Periodontol* 2000 1993;2:57-71.

Grossman A, Ledbetter JA, Rabinovitch PS.Reduced proliferation in T lumphocytes in aged humans is predominatntly in the CD8 subset and is unrelated to defects in transmembrane signaling which are predominantly in the CD4 subset.*Exp Cell Res* 1989.180:367-82.

Kay MMB, Baker Ls.Cell changes associated with declining immune function.*Physiology and cll Biology of Aging*.New York.Raven Press 1972.

Weksler ML.Immune senescence in man.*Immunology and Aging*.Boston.Martinus Nijhoff.1982.165-186

Baker KA, Levy SM, Chrischille EA, Kohout FJ.Medications with dental significance.Use in hursing home population.*Spec Care Dent* 1991.11:19-25.

Race/Ethnicity

The question of whether or not risk for periodontal diseases has anything to do with race or ethnicity is answered by comparing the frequency of disease among groups. Because comparison methodology varies among studies, collective results must be interpreted cautiously. A series of studies examined the data obtained from the National Health and Nutrition Examination Survey III (NHANES III) of 105.8 million civilian non-institutionalized Americans age 30 years and older. Findings have shown that Periodontitis is prevalent in the population, but also point out trends in subpopulations Blacks have the highest prevalence and extent of gingival recession and dental calculus. Mexican Americans have the highest prevalence and extent of gingival gleeding. Mexican Americans had similar prevalence and extent of gingival recession compared with whites. (Albander) Howevere the recession pattern differed between the two groups. Whites and Mexican Americans had 17.5% and 12.9% respectively of buccal surfaces, but comparable percentages of mesial

surfaces with greater than one mm recession. Additionally Mexican Americans showed greater prevalence of attachment loss at mesial than buccal sites. Behavioral, such as differences in oral hygiene habits, rather innate factors may be associated with this observance. (Albandar) .Additionally, attachment loss and destructive periodontitis were consistently more prevalent in males than females and more prevalent in blacks and Mexican Americans that whites.

Similarly aggressive periodontitis prevalence is reportedly higher in blacks than whites. (Melvin). Genetic factors affect aggressive periodontitis risk. Autosomal dominant, recessive and X-linked dominant modes of inheritance have been suggested. (Long). Autosomal dominant inheritance is reported in blacks and whites, but the estimated allele frequency is greater in blacks. However, if aggressive periodontitis is actually a complex multifactorial disease rather than a simple genetically inclined disease, then the allele frequency would be less relevant to the risks.

References

Albandar and Kingman.*Gingival Recession, Gingival bleeding and dental calulus in Adults 30 years of age and older in the united States*jop.1999.70.1.30 1988-1994

Albandar JM, Brunelle JA, Kingman A. Destructiveperiodontal disease in adults 30 years of age and olderin the United States, 1988-1994. *J Periodontol* 1999;70:13-29)

Melvin WL, Sandifer JB, Gray JL. The prevalence and sex ratio of juvenile periodontitis in a young racially mixed population.*J Periodontol* 62:330

Long JC, Nance We, Waring P.Early onset periodontitis.Comparison and evaluation of two proposed modes of inheritance.*Gener Epidemiol*. 4:13. 1987.

Sex

Males tend to have more plaque and gingivitis than females. (Albandar).Additionally, attachment loss and destructive periodontitis were consistently more prevalent in males than females. (Albandar). Due to the mutifactorial nature of periodontal diseases, a single factor such as sex is difficult to associate with disease outcomes. This is especially true when X-linked as well as autosomal inheritance is reported. (Albandar). However, due to hormonal fluctuations during a woman's lifetime women, risks may vary at different times.

Albandar JM, KingmanA.Gingival recession, gingival bleeding and dental calculus in adults 30 years of age and older in the United States.1988-1994.*J periodontal* 70:30, 1999.

Albandar JM, Brunelle JA, Kingman A. Destructiveperiodontal disease in adults 30 years of age and olderin the United States, 1988-1994. *J Periodontol* 1999;70:13-29)

Modifyable Risks for Periodontal Diseases

Socioeconomic Status (SES)

Since socioeconomic status is not innate, and can change during a lifetime, it is considered for this discussion modifiable. Income, occupation and educational level contribute to SES. Periodontal diseases are more prevent and severe in low SES groups. Gingivitis and poor oral hygiene, are more closely related to SES than is periodontitis. Since race, ethnicity and education are components of SES and these factors are often related to that group's access to care. SES may also influence racial difference in periodontal disease prevalence. Periodontitis is most prevalent in blacks, then Mexican American and lastly whites. It is intuitive that the relationship of high SES with better periodontal status is due to better oral hygiene and access to dental care among those groups.

Oral Hygiene

Bacterial plaque biofim is the primary etiology of periodontal diseases, and a necessary trigger for the inflammatory response in gingivitis. Plaque mineralizes to form calculus over the course of a few days; calculus is an irritative agent in the pathogenesis. Since calculus is coated with plaque, it not only serves as an obstable for oral hygiene but also is a plaque reservoir. Because home care is likely not effective in deep pockets, presumably, oral hygiene favorably influenced periodontal outcomes in shallow and moderate pockets and would have little or no effect in deep pockets. In these individuals, periodontal disease is shown to be unrelated to oral hygiene. (Westfelt, Merchant). On the otherhand, it is reported that frequent professional prophylaxis to reach these deeper sites, halts clinical attachment loss. (Axelsson, Axelsson) Persistent bacterial plaque, however is not sufficient to result in periodontal attachment loss. When considering the plaque quantity, week correlations are observed with periodontitis outcomes. (Grossi, Genco) Although qualitative plaque analysis shows that gram-negative anaerobes and "red complex" bacteria are associated with severe periodontitis, recent highly sensitive bacterial studies have identified clonal bacterial subgroups which may or not be pathogenic. (Beck, Listgarten).

In children, perio-pathogens have been identified in supragingival plaque. When left undisturbed, these serve as a reservoir to the subgingival environment. Without professional cleaning and oral hygiene, these pathogens are capable becoming established from childhood. (Tanner, Skellari)

Frequent professional supragingival cleaning,added to good personal oral hygiene, has been shownto have a beneficial effect on subgingival microbiotain moderately deep pockets.

References

U.S. Public Health Service, National Institute of Dental Research.*Oral Health of United States Adults; National Findings.*Bethesda, MD: National Institute of Dental Research; 1987. NIH publication number 87-2868.

Westfelt E. Rationale of mechanical plaque control.*J Clin Periodontol* 1996;23(3 Pt. 2):263-267.

Merchant A, Pitiphat W, Douglass CW, Crohin C, Joshipura K. *Oral hygiene practices and periodontitis in health care*

Axelsson P, Lindhe J. The significance of maintenance care in the treatment of periodontal disease.*J Clin Periodontol* 1981;8:281-294.

Axelsson P, Lindhe J, Nystrom B. On the prevention of caries and periodontal disease.Results of a 15-year longitudinal study in adults.*J Clin Periodontol* 1991;18:182-189.

Grossi SG, Genco RJ, Machtei EE, et al. Assessment of risk for periodontal disease. II. Risk indicators for alveolar bone loss. *J Periodontol* 1995;66:23-29.

Machtei EE, Norderyd J, Koch G, Dunford R, Grossi S, Genco RJ. The rate of periodontal attachment loss in subjects with established

.Beck JD, Koch GG, Zambon JJ, Genco RJ, Tudor GE.Evaluation of oral bacteria as risk indicators for periodontitis in older adults.*J Periodontol* 1992;63:93-99.

Listgarten MA. Microbiological testing in the diagnosis of periodontal disease.*J Periodontol* 1992;63(Suppl.): 332-337.

Listgarten MA, Slots J, Nowotny AH, et al. Incidence of periodontitis recurrence in treated patients with and without cultivable *Actinobacillus actinomycetemcomitans*, *Prevotella intermedia*, and *Porphyromonas gingivalis*: A prospective study. *J Periodontol* 1991;62: 377-386.

Wennström J, Dahlen G, Svensson J, Nyman S. *Actinobacillus actinomycetemcomitans, Bacteroides gingivalis* and *Bacteroides intermedius*; predictors of attachment loss?

Tanner AC, Milgrom PM, Kent RJ, et al.The microbiota of young children from tooth and tongue samples.*J Dent Res* 2002;81:53-57.

Sakellari D, Belibasakis G, Chadjipadelis T, Arapostathis K, Konstantinidis A. Supragingival and subgingival microbiota of adult patients with Down's syndrome. Changes after periodontal treatment.*Oral Microbiol Immunol* 2001;16:376-382.

Systemic Risk Factors

DiabetesMellitus

Patients with poor glycemic control either due to undiagnosed or poorly controlled Type I or II diabetes are susceptible to periodontal diseases. (Papapanou, Mealey) Diabetes, a dysregulation in carbohydrate, lipid and protein metabolism, increases the risk of periodontal diseases a number of potential ways, but is difficult to define conclusively. Immune cell function is altered in diabetics. (AmericanAcademy of Periodontology) Neutrophil function is reduced, TNF-alpha response is increased. Therefore, even when similar sub-gingival microflora are present, diabetics present with more severe, progressing periodontal destruction. Because evidence suggests that the same microvascular complications that that contribute to the classic complications of diabetes, retinopathy, nephropathy, neuropathy,and impaired wound healing, also increase the risk for periodontal diseases, it has been suggested that periodontitis should be added to the list. Microvascular changes which characterize diabetic patients include abnormal growth and impaired regeneration. Advanced glycation

end products (AGE) accumulate in patients with hyperglycemia. These proteins impact cell-cell and cell-matrix interactions. AGEs accumulate on vessel walls and contribute to characteristic changes which resemble atherosclerosis. As endothelial cell basement membranes thicken, the lumen narrows; vascular permeability and thrombus formation occurs. (Wautier) When AGEs bind to receptor, called RAGE, proinflammatory cytokine production increases. Proinflammatory cytokines, IL-1Beta, TNF-a, prostaglandin E2, intern stimulate the inflammatory process which contributes to breakdown of collagen, bone and the structural components of the periodontium. Compounding greater tissue destruction, altered connective tissue metabolism in hyperglycemia results in decreased osteoblastic, collagen cell and fibroblast production. (Stuart, Inaba, Tisdel) Decreased proliferation coupled with increased destruction of periodontal structures results in greater risk for developing periodontal diseases, and also effects therapeutic response. Some research shows post-therapeutic improvement in periodontal parameters, poor glycemic control has been associated with recurrence and poor longterm outcomes. (Tervonen).

Just as poor glycemic control effects the periodontium, it is suggested that periodontal disease can impact the glycemic state as well. Periodontitis is shown to increase the risk of poorer glycemic control. (Taylor) Additionally, after periodontal therapy, significantly better glycemic control has been noted with clinically evident improvement in periodontal parameters. Periodontal inflammation may induce or perpetuate a systemic chronic inflammatory state (Loos). Such a state increases insulin resistance and can negatively effect glycemic homeostasis. (Genco). For example, periodontitis patients present with higher serum inflammatory markers, C-reactive protein (CRP), Il-6, and fibrinogen. (Loos) The aim of periodontal therapy is reduce inflammation, and therapy is shown to result in decrease in these markers. By virtue of this reduction in systemic inflammation, periodontal therapy impacts the systemic environment.

References

Papapanou PN. Periodontal diseases: Epidemiology. *Ann Periodontol* 1996;1:1-36.

Mealey BL, Moritz AJ. Hormonal influences: Effects of diabetes mellitus and endogenous female sex steroid hormones on the periodontium. *Periodontol*2000 2003;32:59-81.

Wautier J-L, Guillausseau P-J. Diabetes, advanced glycation endproducts and vascular disease. *Vasc Med* 1998;3:131-137.

White CB, Turner NS, Lee GC, Haidukewych GJ. Open ankle fractures in patients with diabetes mellitus. *Clin Orthop* 2003;414:37-44.

Stuart MJ, Morrey BF. Arthrodesis of the diabetic neuropathic ankle joint.*Clin Orthop* 1990;253:209-211.

Inaba M, Nishizawa Y, Mita K, et al. Poor glycemic control impairs the response of biochemical parameters of bone formation and resorption to exogenous 1,25-dihydroxyvitamin D3 in patients with type 2diabetes. *Osteoporos Int* 1999;9:525-531.

Tisdel CL, Marcus RE, Heiple KG. Triple arthrodesis for diabetic peritalar neuroarthropathy. *Foot Ankle Int* 1995;16:332-338.

Tervonen T, Karjalainen K. Periodontal disease related to diabetic status. A pilot study of the response to periodontal therapy in type 1 diabetes. *J Clin Periodontol* 1997;24:505-510.

Taylor GW, Burt BA, Becker MP, et al. Severe periodontitis and risk for poor glycemic control in patients with non-insulin-dependent diabetes mellitus. *J Periodontol* 1996;67:1085-1093.

Loos BG. Systemic markers of inflammation in periodontitis.*J Periodontol* 2005;76:2106-2115.

Genco RJ, Grossi SG, Ho A, Nishimura F, Murayama Y. A proposed model linking inflammation to obesity, diabetes and periodontal infections.*J Periodontol* 2005;76:2075-2084.

Loos BG, Craandiji J, Hoek FJ, Wertheim-van Dillen PME, van der Velden U. C-reactive protein and other markers of systemic inflammation in relation to cardiovascular diseases are elevated in periodontitis. *J Periodontol* 2000;71:1528-1534.

Systemic inflammation is significantly elevated in diabetes and conditions of poor glycemic control, but also in obesity, insulin resistance. High CRP and fibrinogen is identified in obese and insulin resistant patients. (Haffner). Obesity reflects a change in normal metabolic and endocrine adipose function through an increased production of fatty acids cytokines and acute phase proteins; in fact the condition has been called a metabolic syndrome. Adipose tissue has endocrine function through the productions of hormones such as leptin, resistin and adiponectin which regulate appetitie, blood pressure, angiogeneis and most notably insulin sensitivity and immune function (Ronti, Nishida). These conditions are considered chronic inflammatory states and bear pathophysiologic similarities to athersclerosis. All of these conditions are linked to proinflammatory cytokines IL6, and TNF-a. (Bissada) Although the physiopathologic mechanisms are not fully understood, insulin sensitivity, the role of adipkines, genes, environment and inflammatory mediators, inflammatory conditions such as periodontal diseases may also play a role. Obesity is associated with increased risk for periodontal dieases. (Alzahrani) Their treatment warrants particular considerations.

References

Haffner S, Temprosa M, Crandall J, et al. Intensive lifestyle intervention or metformin on inflammation and coagulation in participants with impaired glucose tolerance. *Diabetes* 2005;54:1566-1572.

Ronti T, Lupattelli G, Mannarino E. The endocrine function of adipose tissue: An Wood N, Johnson RB, Streckfus CF. Comparison of body composition and periodontal disease using nutritional assessment techniques: Third National Health and Nutrition Examination Survey (NHANES III). *J Clin Periodontol* 2003;30:321-327.

Nishida N, Tanaka M, Hayashi N, et al. Determination of smoking and obesity as periodontitis risks using the classification and regression tree method. *J Periodontol* 2005;76:923-928.

Obesity and periodontal Bissada NF.Quintessence Int. 2006 Nov-Dec;37(10):751.

Periodontitis and three health

Al-Zahrani MS, Borawski EA, Bissada NF. *J Periodontol*. 2005 Aug;76(8):1362-6.

Hormonal Fluctuations in Women

Hormonal changes associated with puberty, menstrual cycle, pregnancy and menopause, are known to impact periodontal diseases. Hormone changes in pregnancy warrant treatment considerations. Gingival erythem, edema, bleeding and hyperplasia characterize a condition which was first identified as“pregnancy gingivitis” in the late 1800’s. (Loe). Figure 29 Although the clinical features resemble gingivitis, the etiologic factors are related to hormone related immunologic changes related to pregnancy. Reportedly, increased prostaglandins depress immune response to a differing microbial composition and increased ovarian progesterone production stimulates inflammation. Additionally, pyogenic granulomas, sometimes called “pregnancy tumor”, occur in 1-10% of pregnancies. (Bhashkar). They appear as tumorlike growths generally located on the interdental papillae in the maxillary anterior.

Figure 29.

Oral contraceptives are exogenous ovarian hormones; their effect on the periodontium is similar to that of pregnancy. Inflammation may be increased and there is an exagerated response to irritants. Salivary composition protein sialic acid, hydrogen ions and electrolites has been shown.

Menopause is marked by a rapid decline in estrogen. As estrogen declines in menopause, so do the skeletal bones and some data suggests, so do teeth. 1 The rate of bone loss in postmenopasal women predicts tooth loss: for every 1% per year decrease in whole-body bone mineral density, the risk for tooth loss increases more than four times. 2 Studies examining the relationship between estrogen status and periodontal disease report suggest estrogen plays an important role indetermining mandibular bone mass and in modifying the severity of periodontal disease in postmenopausal women.

Medications which alter hormone levels such as oral contraceptives, and post-menopausal hormone replacement therapy (HT) also impact periodontal health. Postmenopausal women who do not use HT had a greater chance of having periodontitis than premenopausal women. In contrast, postmenopausal using HT and premenopausal women had similar periodontal status. HT may have a beneficial effect on periodontal health.Studies suggest that hormone therapy confers protection against tooth loss and reduces the risk for edentulism. Two cross-sectional studies in the US have shown tooth loss risk in older postmenopausal women using estrogen, is significantly reduced compared to those who were not.Additionally, a longitudinal study by Grodstein et al, an inverse relationship between estrogen therapy and tooth loss was reported.On the contrary,Taguchi et al evaluated over 300 postmenopausal Japanese women and found no significant difference in the number of total teeth between estrogen users and non-users. This may be explained by the younger population in this study compared to the other two cross-sectional studies mentioned. The duration of estrogen use though was significantly associated with the total number of teeth remaining independent of age.

Although the relationship between hormone status and periodontal disease is complex and remains to be fully understood, the preponderance of evidence warrants considerating hormone fluctuation and its risks for periodontal diseases.

References

Bhashkar S, Jacoway JR.Pyogenic granuloma:clinical features, incidence, histology, and results of treatment.Report of 242 cases.*J Oral Surg* 1966;24:391-8.).

Loe H, SilnessJ.Periodontal disease in pregnancy.Prevalence and severity.*Acta OdontolScand* 1984;21:51-5

North American Menopause Society.*Menopause Practice: A Clinician's Guide, 3rd Edition*, 2007.

Krall EA, GarciaRI, Dawson-Hughes B. Increased risk of tooth loss is related to boneloss at the whole body, hip and spine. *Calcif Tissue Int* 1996;59:433-437

J Periodontol. 2009 Sep;80(9):1380-7.Association among menopause, hormone replacement therapy, and periodontal attachment loss in southern Brazilian women.Haas AN, Rösing CK, Oppermann RV, Albandar JM, Susin C.Genco RJ, Grossi SG. Is estrogen deficiency a risk factor for periodontal disease?*Compend Contin Educ Dent Suppl* 1998; 22:S23-9.

Brennan RM, Genco RJ, Wilding GE, et al. Bacterial species in subgingival plaque andoral bone loss in postmenopausal women. *J Periodontol* 2007; 78(6):1051-1061.

Paganini-Hill A. The benefits of estrogen replacement therapy on oral health.*Arch Intern Med* 1995; 155:2325-2329.

Krall EA, Dawson-Hughes B, Hannan MT, et al. Postmenopausal estrogen replacementand tooth retention. *Am J Med* 1997; 1C2:536-542.

Grodstein F, Colditz GA, Stampfer MJ, et al. Postmenopausal hormone use and toothloss: a prospective study. *J Am Dent Assoc* 1996; 127:370-377.

Drug Induced

Anticonvulsants, calcium channel blockers, and cyclosporine are associated with gingival enlargement. Gingival enlargement occurs in only about one-third of patients using these medications, and is more prevalent in patients with poor plaque score. Inflammation secondary to poor plaque control can progress to attachment loss. In the presence of gingival overgrowth examining for the attachment level through periodontal probing and establishing clinical attachment loss is more technique sensitive. Oral contraceptives are associated with increased vascular permeability and inflammation as mentioned above. Since medications taken for systemic disease impact the periodontium, they impact risks for developing and progression of gum diseases.

Hematologic Disorders

Hemmorrhagic gingival enlargement is a common early sign of acute leukemia. It may occur with or without necrosis. In chronic leukemia, periodontal changes are similar but less severe. Chemotherapy or immune suppressant, anti-rejection mediations associated with bone marrow transplant may also impact the periodontium.

Immune System Disorders

Primary and secondary immune suppression impacts the periodontium. The host response perio-pathogens is reduced. In primary immune suppression conditions such as developmental, and HIV, severe form s of periodontal diseases are observed. Necrotizing diseases and fungal infections are more prevalent . Secondary immune suppression occurs in patients in organ transplant, and cancer patients.

Osteoporosis

Osteoporosis, a chronic skeletal disorder characterized by compromised bone strength and predisposing a person to increased fracture risk, is a common condition. The annual incidence of osteoporosis-related fractures is estimated at over 2million; the annual costs of treating these patients is estimated at 13 billion dollars. (Burge). Although it s a costly and widespread condition, osteoporosis is largely undertreated; particularly among postmenopausal women, NHANES data suggest that despite having two or more risk factors for ostoporosis, only a minority use bone therapeutic medication. (Gehlbach) The condition principally affects postmenopausal women but also occurs in men and pre-menopausal women. It can occur secondary to medication such as glucocorticord therapy.

Osteoporosis and periodontitis are both chronic diseases with an increased prevalence in aging populations. In both conditions, local macrophage and subsequent cytokine production, including interleukin 1,6,8, and 10 and tumor necrosis factor alpha (TNF-a), enhance

osteoclast mediated bone resorption. Longitudinal studies have questioned the relationship between systemic bone loss, periodontal disease and edentulism, (Famili) however the AmericanAcademy of Periodontology considers osteoporosis a risk factor for periodontal disease.

References

Burge R et al. *J Bone Miner Res.* 2007;22(3):465-475.White women age ≥65 years and taking antiresorptive medications.

Gehlbach SH et al. *Osteoporos Int.* 2007;18:805-810.

Famili P, Cauley J, Suzuki JB, et al. Longitudinal study of periodontal disease and edentulism with rates of bone loss in older women. *J Periodontol* 2005; 76(1):11-15.

American Dental Association Council on Access, *Prevention and Interprofessional Relations. Women's Oral Health Issues*. Nov 2006.

Lifestyle Choices as Risk Factors

Smoking and Tobacco

Smoking is one of the strongest risk factors in the development and progression of periodontal diseases and subsequent tooth loss. Since the 1940's, smoking has been associated with specific types of periodontal diseases such as Acute necrotizing ulcerative gingivitis (ANUG). (Pindborg) Studies show smokers have greater attachment and bone loss, more deep pockets, and greater calculus formation. Smokers have a 2.5-6.0 times greater risk depending on which periodontal parameter is measured. Higher prevalence of furcation is noted among smokers and smokers had nearly double radiographic evidence of furcation involvement than never smokers. (Axelsson) Alveolar bone loss is greater in smokers and average alveolar bone height as a percentage of root length is significantly lower. (Bergstrom Eliasson)

Smoking effects the host response to periopathogenic attack by compromising neutrophil function. Impaired chemotaxis and phagocytosis are reported. Nicotine exposure inhibits neutrophil production of superoxide anion and hydrogen peroxide. (Kenney)

Smoking contributes to destruction of surrounding healthy periodontal tissue In shallow pockets, smoking appears to promote colonization of perio-pathogenic bacteria. (Haffajee) Smoking alters host repoonse in inflammatory diseases by increasing TNF-a production and cytokine release. (Gustoaffson, Fredrikkson) Host response to periodontal pathogens is altered as smoking inhibits granulocyte function. Additionally, interactions between gene clusters and smoking have been identified. Vasoconstriction present in smokers, tends to mask inflammation in gingivitis. (Bergstrom).

Tobacco use also reduces the response to periodontal therapy. Smoking is a major predictor for response to periodontal therapy. Smokers are twice as likely to lose teeth while on maintenance therapy following (McGuire and Nunn) active periodontal therapy. Additionally, smokers were mokre likely to show less reductions in probing depth, clinical

attachment gains. (Ah) PDL Fibroblasts in smokers show inhibition of growth and attachment. Additionally, smokers show slower post treatment reduction of white cells and neutrophils. Reportedly, 90% of refractory chronic periodontitis cases are in smokers. (James)

References

Bergstrom J, Preber H. Tobacco use as a risk factor. *J Periodontol* 1994;65(Suppl.):545-550.

Pindborg JJ. Tobacco and gingivitis. I. Statistical examination of the significance of tobacco in the development of ulceromembranous gingivitis and in the formation of calculus. *J Dent Res* 1947;26: 261-264.

Pindborg JJ. Tobacco and gingivitis. II. Correlation between consumption of tobacco, ulceromembranous gingivitis and calculus. *J Dent Res* 1949;28:460-463.

Axelsson P, Paulander J, Lindhe J. Relationship between smoking and dental status in 35-, 50-, 65-,and 75-year-old individuals. *J Clin Periodontol* 1998;25:297-305.

Mullally BH, Linden GJ. Molar furcation involvement associated with cigarette smoking in periodontal referrals. *J Clin Periodontol* 1996;23:658-661.

Bergström J, Eliasson S. Cigarette smoking and alveolar bone height in subjects with high standard of oral hygiene. *J Clin Periodontol* 1987;14:466-469.

Kenney EB, Kraal JH, Saxe SR, Jones J. The effect of cigarette smoke on human oral polymorphonuclear leukocytes.*J Periodont Res* 1977;12:227-234.

Haffajee AD, Socransky SS. Relationship of cigarette smoking to the subgingival microbiota. *J Clin Periodontol* 2001;28:377-388.

Gustafsson A, Asman B, Bergstrom K. Cigarette smoking as an aggravating factor in inflammatory tissuedestructive diseases. Increase in tumor necrosis factor-alpha priming of peripheral neutrophils measured as generation of oxygen radicals. *Int J Clin Lab Res* 2000;30:187-190.

Fredriksson M, Bergstrom K, Asman B. IL-8 and TNF-□from peripheral neutrophils and acute-phase proteins in periodontitis. *J Clin Periodontol* 2002;29:123-128.

McGuire MK, Nunn ME. Prognosis versus actual outcome. III. The effectiveness of clinical parameters in accurately predicting tooth survival. *J Periodontol* 1996;67:666-674.

Ah MKB, Johnson GK, Kaldahl WB, Patil KD, Kalkwarf KF. The effect of smoking on the response to periodontal therapy.*J Clin Periodontol* 1994; 21:91-97.

Kaldahl WB, Johnson GK, Patil KD, Kalkwarf KL. Levels of cigarette consumption and response to periodontal therapy.*J Periodontol* 1996;67:675-681.

James JA, Sayers NM, Drucker DB, Hull PS. Effects of tobacco products on the attachment and growth of periodontal ligament fibroblasts.*J Periodontol* 1999;70: 518-525.

Christan C, Dietrich T, Hagewald S, Kage A, Bernimoulin JP. White blood cell count in generalized aggressive periodontitis after non-surgical therapy.*J Clin Periodontol* 2002;29:201-206.

Coping with Stress

Stress and related psychological disorders associated with maladaptive coping mechanisms have been associated with oral pathology including periodontal diseases. When the association of stress, distress and coping behaviors with periodontal disease was assessed, stress associated with financial strain and distress manifest as depression are significant risk indicators for more severe periodontal disease. Adequate coping behaviors may reduce the stress-associated risk, but further investigation is needed to to explain the relationships.

References

Relationship of Stress Distress and inadequate coping behaviors to periodontal disease.Genco RJ, Ho AW, Grossi SG, Dunford RG and Tedesco LA.*J periodontol* 1999; 70 711-23.

Surgical Therapy

Periodontal surgery is any surgical procedure used to treat periodontal disease or to modify the periodontal morphology.The goal of surgery is to restore health, comfort, esthetics and function.

Resective Surgery

Resective surgery aims to reduce progressing periodontal destruction. By apically positioning a mucoperiosteal flap, periodontal pocketing can be eliminated with minimal loss of keratinized tissue. In the presence of periodontal pockets, which persist beyond successful initial non-surgical therapy and oral hygiene compliance due to unfavorable underlying boney architecture, removal of the soft tissue component of the pocket, i.e. gingivectomy, will fail if the underlying bone morphology is not reshaped to support the soft tissue architecture. Osseous surgery reshapes alveolar bone in order to create bony architecture which is compatible with physiologic architecture. (Schluger). Additionally, when alveolar bone morphology is irregular or when wide, saucer shaped, interproximal pockets, in which regeneration is not predictable are indications for osseous surgery. On the contrary, defects with thick bony ledges, or when pocket elimination will result in a sacrifice tissue on adjacent teeth osseous surgery is contraindicated. Figure 30. The main advantage disadvantage of osseous surgery is loss of attachment. (Siebert) In classic periodontal literature a palatal approach to osseous surgery is advocated to remove osseous craters present along maxillary teeth in order to preserve keratinized tissue on the buccas surface, because of greater access to palatal embrasures, the cleansing effect of the tongue and less post-surgical bone loss. . (Ochsenbein) Similarly, a lingual approach was suggested in the mandible to avoid thick buccal bone shelves, take advantage of longer lingual root trunks and the location of the lingual furcation, and the creation of better post-surgical access for hygiene. (Tibbits).

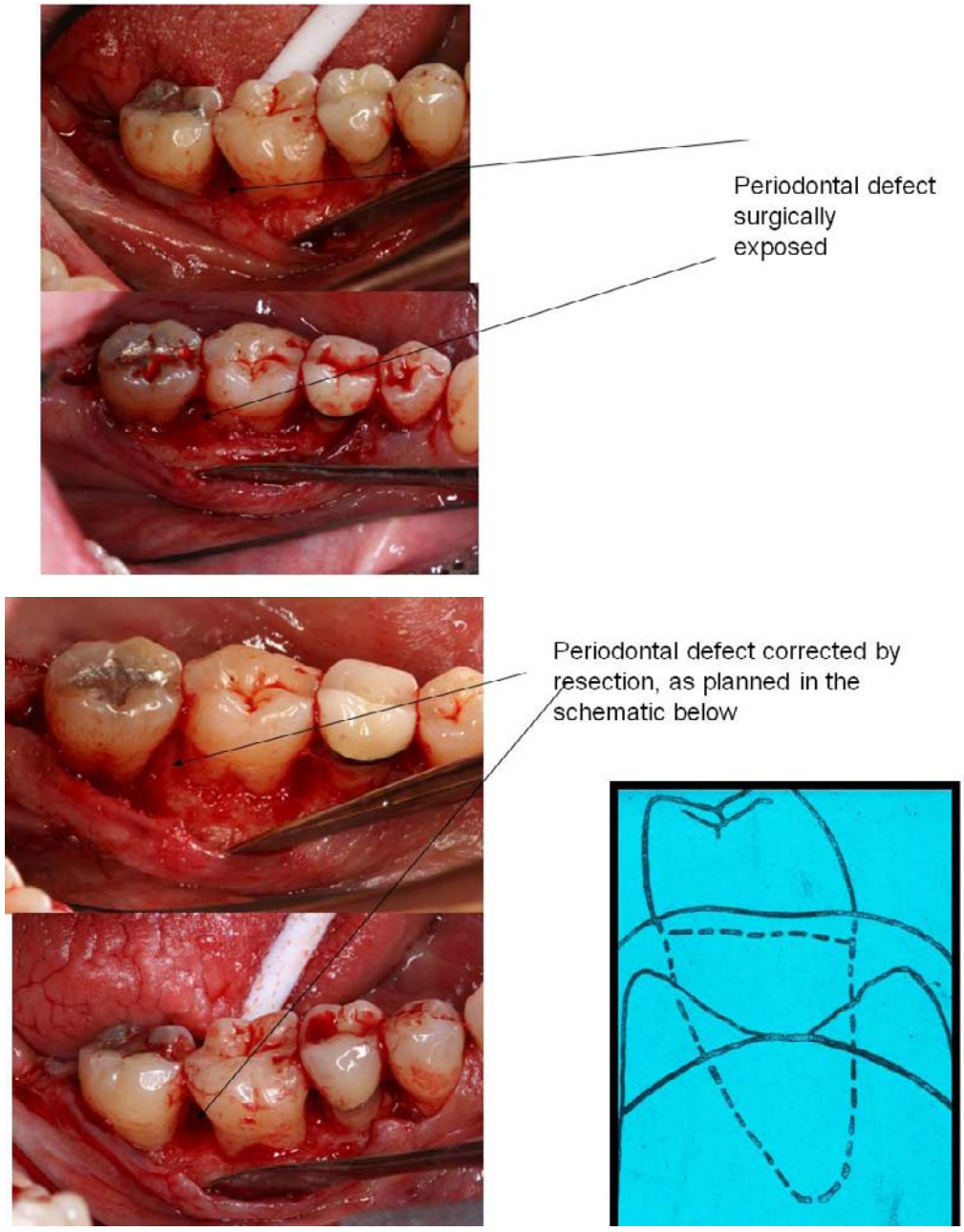


Figure 30.

Root resection during resective surgery may be useful if severe bone loss, root fracture, caries or resorption does not affect all of a tooth's roots or if class III furcation, a lesion in which regeneration is not predictable, exists. Because endodontic therapy must have been completed prior to root resection, it is considered a costly procedure and often accompanied by tooth extraction and replacement with a dental implant in treatment options.

References

Schluger S. Osseous resection—a basic principle in periodontal surgery.*Oral Surg Oral Med Oral Pathol* 1949;2:316-325.

Siebert J. Treatment of infrabony lesions by surgical resection procedures. In: Stahl SS, ed. *Periodontal Surgery: Biologic Basis and Technique.* Springfield, IL: Charles C. Thomas; 1976.

Ochsenbein C, Bohannan H. The palatal approach to osseous surgery. I.Rationale. *J Periodontol* 1963;34:60-68.

Tibbetts L, Ochsenbein C, Loughlin D. Rationale for the lingual approach to mandibular osseous surgery. *Dent Clinics N Am* 1976;20:61-78.

Regenerative Surgery

Regeneration is the reproduction or reconstitution of a lost or injured part. This is in contrast to repair which is the healing which does not fully restore the architecture or function. (AAP Glossary). Periodontal regeneration refers to the histologic regeneration of the periodontal attachment including alveolar bone, PDL and cementum associated with a previously diseased root. New attachment, epithelial and/or connective tissue, forms in the case of regeneration whereas reattachment describes the reunion of epithelial and connective tissue healing following resective surgery. (AAP Glossary) The therapeutic goal of regenerative surgery, then in to generate new alveolar bone, PDL, and cementum, even though radiographically only the new bone is visible and clinically new attachment cannot be distinguished from reattachment. Figure 31. Bone replacement grafts, autografts and allografts, show strong clinical outcomes with various types of membranes, (Reynolds) and since research is constantly progressing, other materials are being introduced for specific indications. For instance enamel matrix derivative, bone morphogenic proteins, and platelet rich plasma are expanding regeration predictability. (Sallum, Schallhorn, Schallhorn, Schallhorn, Burnette, Schallhorn, Froum) Specific uses and indications are beyond the scope of this chapter.

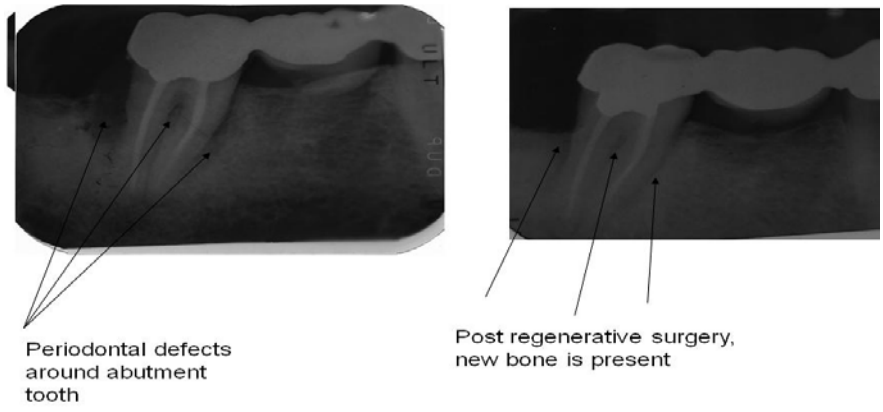


Figure 31.

References

American Academy of Periodontology.*Glossary of Periodontal Terms*.Chicago: American Academy of Periodontology; 2001.

Reynolds MA, Aichelmann-Reidy ME, Branch-Mays GL, Gunsolley JC. The efficacy of bone replacement grafts in the treatment of periodontal osseous defects. A systematic review.*Ann Periodontol* 2003;8:227-265.

287. Sallum EA, Casati MZ, Caffesse RG, Funis LP, Nociti Junior FH, Sallum AW. Coronally positioned flap with or without enamel matrix protein derivative for the treatment of gingival recessions. *Am J Dent*

Schallhorn RG. Eradication of bifurcation defects utilizing frozen autogenous hip marrow implants. *J Ont DentAssoc* 1968;45:18-22.

Schallhorn RG. The use of autogenous hip marrow biopsy implants for bony crater defects. *J Periodontol* 1968;39:145-147.

Schallhorn RG, Hiatt WH, Boyce W. Iliac transplants in periodontal therapy.*J Periodontol* 1970;41:566-580.

Burnette EW Jr. Fate of an iliac crest graft.*J Periodontol* 1972;43:88-90.

Schallhorn RG, Hiatt WH. Human allografts of iliac cancellous bone and marrow in periodontal osseous defects. II. Clinical observations. *J Periodontol* 1972;43:67-81.

Froum SJ, Ortiz M, Witkin RT, Thaler R, Scopp IW, Stahl SS. Osseous autografts. III. Comparison of osseous coagulum-bone blend implants with open curetage. *J Periodontol* 1976;47:287-294.

Plastic Surgical Procedures

In cases of with unfavorable periodontal architecture either for ideal esthetics or for planned restorations, gingival recession, or alveolar ridge defect, surgical molding of tissue is used to improve the condition. In the case of a gummy smile (due to altered passive eruption or gingival overgrowth) or partially erupted teeth, when excess gingival exposure is present, or when biologic width of attachment interferes with a planned restorative margin, clinical crown lengthening can be indicated. Clinical crown lengthening generally involves apically positioning the gingiva and may be done with or without osseous recontrouring, and generally aims to preserve the papilla along with keratinized tissue (Coslet, Tarnow). Figure 32. In the case of recession, several surgical approaches are useful in different applications. For instance,laterally positioned flap , rotational flap such as double papilla oblique rotated and papilla rotation flap (Cohen, Ross, Pennel) techniques are useful to cover the exposed root surface. (Grupe, Grupe) In the 1980's subepitelial connective tissue graft for root coverage gained popularity due to its high predictability.(Langer, Langer) Figure 33. Newer techniques allow for mimimal surgical trauma, by using donor tissue, tunnel preparation, and microsurgical techniques. (Harris, Rosetti) Enamel matrix derivatives are shown to enhance root coverage.(Abbas) In the case of alveolar ridge defects, either when teeth are missing and implant restoration in planned or when teeth are present and fixed or removable fixed partial denture is planned, bone and soft tissue augmentation can be indicated. Alveolar ridge aumentation, connective tissue grafting or components of the two together are applicable

often with enamel matrix derivative, bone morphogenic proteins, or platelet rich plasma. Surgical techniques are beyond the scope of this chapter. (Marx, Vastardis)

Figure 32.

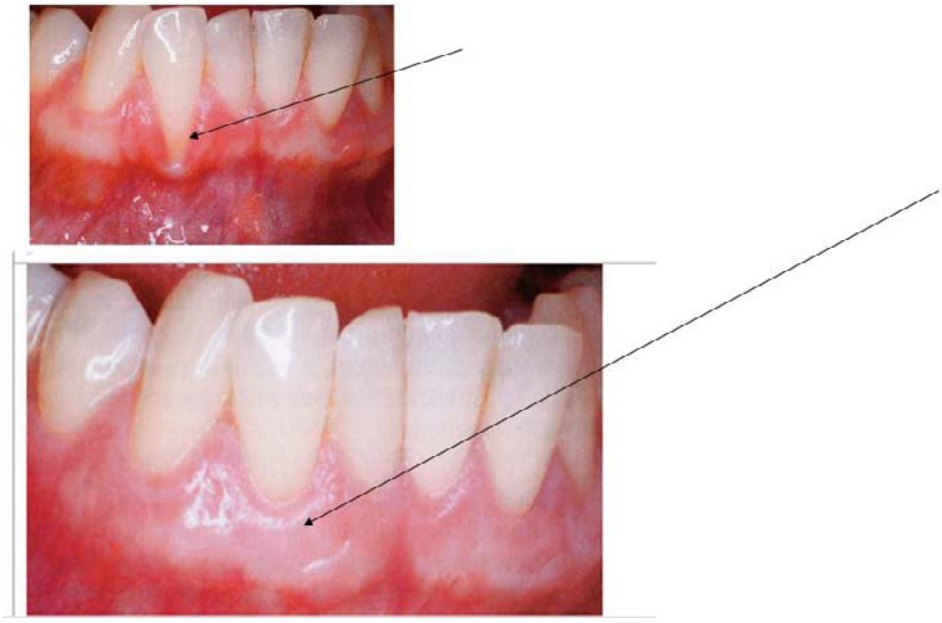


Figure 33.

References

Coslet JG, Vanarsdall RL, Weisgold A. Diagnosis and treatment of delayed passive eruption of the dentogingival junction in the adult. *Alpha Omegan* 1977; 70:24-28.

Tarnow D, Magner A, Fletcher P. The effect of the distance from the contact point to the crest of bone on the presence or absence of the interproximal dental papilla.*J Periodontol* 1992;63:995-996.

Grupe HE, Warren RF Jr. Repair of gingival defects by a sliding flap operation.*J Periodontol* 1956;27:92-95. Grupe HE.Horizontal sliding flap operation.*Dent Clin North Am* 1960;4:43-46.

Cohen DW, Ross SE. The double papillae repositioned flap in periodontal therapy. *J Periodontol* 1968;39:65-70.

Ross SE, Crosetti HW, Gargiulo A, Cohen DW. The double papillae repositioned flap – an alternative. I. Fourteen years in retrospect. *Int J Periodontics Restorative Dent* 1986;6(6):46-59.

Pennel B, Higgason J, Towner J, King K, Fritz B, Sadler J. Oblique rotated flap. *J Periodontol* 1965;36:305-309.

Langer B, Calagna LJ. The subepithelial connective tissue graft.A new approach to the enhancement of anterior cosmetics.*Int J Periodontics Restorative Dent* 1982; 2(2):22-33.

Langer B, Langer L. Subepithelial connective tissue graft technique for root coverage. *J Periodontol* 1985;56:715-720.

Harris RJ. A comparative study of root coverage obtained with an acellular dermal matrix versus a connective tissue graft: Results of 107 recession defects in 50 consecutively treated patients. *Int J Periodontics Restorative Dent* 2000;20:51-59.

Rosetti EP, Marcantonio RA, Rossa C Jr, Chaves ES, Goissis G, Marcantonio E Jr. Treatment of gingival recession: Comparative study between subepithelial connective tissue graft and guided tissue regeneration. *J Periodontol 2*000;71:1441-1447.

Abbas F, Wennström J, van der Weijden F, Schneiders T, van der Velden U. Surgical treatment of gingival recessions using Emdogain gel: Clinical procedure and case reports. *Int J Periodontics Restorative Dent* 2003;23:607-613.

Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, GeorgeffK.Platelet rich plasma—Growth factor enhancement for bonen grafts.Oral Surg Oral Med Oral Patholo *Oral Radiol Endod* 1998;85:638-46.

Vastardis S, Yukna RA, Mayer ET. Platelet rich plasma plus Alloderm for gingival recession treatment .*J Dent Res* 2004; 83.

Dental Implant Placement

The therapeutic goal of dental implant therapy is to support restorations that replace a tooth or missing teeth in order to provide comfort, function and esthetics (AAP Statement). Studies have shown the success of dental implants. There are no absolute contraindications for dental implant placement. Several relative contraindications are known to a risk for dental implant success, although periodontitis or a strong susceptibility to periodontitis is not one of them. As a result, patients with history of periodontitis, or at risk for developing and

progressing periodontal diseases, are candidates for dental implants. (Nevins) Dental implant cases begin with presurgical evaluation. Restorative parameters such as space available for the crown, and esthetics are considered by evaluating casts, photographs and bite registration. Imaging is used to evaluate size and shape and of available bone, and address limitations of local anatomy such as Maxillary sinus, foramen, inferior alveolar nerve canal and adjacent teeth. Cone-beam ct (CBCT) can give useful 3-dimensional information about the location of these structures, such pre-surgical imaging can be used to simplify surgical procedures and is discussed in the section on CBCT. When inadequate bone quality or limitation due to local anatomy is identified, the site must be prepared prior to dental implant placement. Figure 34. Several augmentation strategies, such as maxillary sinus augmentation, ridge block grafting, and expansion can be used to create a more ideal site. The implant placement procedure aims to minimize thermal trauma to bone, permit osseointegration and limit micromotion during healing. Recently, early and immediate implant loading has shown promise. Thermal trauma is reduced by using externally and internally irrigated drills with higher speed lower torque handpieces. (Erickson). Since no difference in success rates are shown for sterile, versus "clean" conditions, and sterile conditions are difficult if not impossible to maintain during intra-oral procedures, surgical placement of dental implants under aseptic conditions is sufficient. (Scharf) A surgical template may be useful.

Dental implant placement at the time of tooth extraction, immediate placement, is shown to be successful when rigid fixation is achieved. Peri-implant tissues can be maintained for comfort and esthetics in this way. (Gher, Gelb, Schwartz) On the other hand, dental implant placement can occur after the extraction socket is healed, usually a period of 3 months. (Tarnow). When implant placement is planned after extraction, maintaining bone quality and quality in the alveolar ridge is critical. Dental extractions result in an alveolar bone defect through normal healing when the fibrin rich clot retracts and connective tissue invades the socket before bone forms there. In this situation the socket collapses resulting in a bone defect. Such a condition in the anterior maxilla, where buccal and palatal bone cortices are thin, compromises implant esthetics. Different surgical strategies to avoid bone loss during extraction can help to preserve the needed bone. Such strategies include interdental papilla preservation, use of instruments, such as periotomes. Because research suggests that resorption occurs regardless of trauma during the extraction, ridge preservation at the time of extraction is designed to preserve critical tissue. (Lorenzoni).

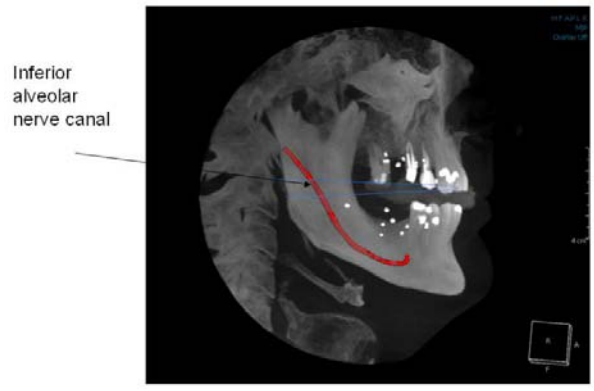


Figure 34.

Although noted as being highly successful, several complications have been reported. These include fixture mobilityor fracture, inflammation or peri-implantitis with associated progressing loss of peri-implant tissues, pain, paresthesia and neuropathy. Restorative complications may include prosthesis instability (which may be related to loosening components), and occlusal trauma. (Rosenquist, Tarnow)

References

Parameter on placement and management*J Periodontol* 2000;71:870-872.

Nevins M, Langer B. The successful use of osseointegrated implants for the treatment of the recalcitrant periodontal patient. *J Periodontol* 1995;66:150-157.

REFMarginal boneMa S, Payne AG.*Int J Prosthodont*. 2010 Mar-Apr;23(2):117-26. Review.

Erikson A, Adell R. Temperatures during drilling for the placement of implants using the osseointegration technique. *J Oral Maxillofac Surg* 1986;44:4-7.

Scharf D, Tarnow D. Success rates of osseointegration for implants placed under sterile versus cleanconditions. *J Periodontol* 1993;74:954-956.

Gher ME, Quintero G, Assad D, Monaco E, Richardson AC. Bone grafting and guided bone regeneration for immediate dental implants in humans.*J Periodontol* 1994;65:881-891.

Gelb DA. Immediate implant surgery: three-year retrospective evaluation of 50 consecutive cases. *Int J Oral Maxillofac Implants* 1993;8:388-399.

Schwartz-Arad D, Ghaush G. Full-arch restoration of the jaw with fixed ceramometal prostheses. *Int J Oral Maxillofac Implants* 1998;13:819-825.

Lorenzoni M, Pertl C, Polansky RA, Jakse N, Wescheider WA.Evaluation of implants placed with barier membranes.Clin Oral Implants Res 2002:13:274-80.

Rosenquist B, Grenthe B. Immediate placement of implants into extraction sockets: implant survival. *Int J Oral Maxillofac Implants* 1996;11:205-209.

Tarnow DP, Fletcher P. The two-to-three month postextraction placement of root form implants: a useful compromise. *Implants: Clin Rev Dent* 1993;2:1-6.

Long Term Maintenance

Osseointegration is defined as a direct structural connection between bone and the surface of a load-bearing implant. (Branemark). Unlike a healthy tooth, which is attached to bone through PDL, dental implant is osseo integrated to bone in a fashion similar to an ankylosed tooth. Long term dental implant care warrants special consideration.

Proper dental implant maintenance is needed for long term success (Chung). Just as bacterial plaque biofilm is the causative agent for gingivitis and periodontitis, it also induces peri-implantitis Oral hygiene and plaque scores are useful to assess home care effectiveness, and are critical when natural teeth, which may be affected by periodontal disease could act as a reservoir for perio-pathogens. (Heitz) Regular clinical examination for signs of inflammation, bleeding on probing, exudate are needed. Prosthetic, implant, and neighboring tooth structures should be evaluated for plaque and calculus. Stability of the dental implant

should be checked. Mobility is the best indicator for implant failure. Unlike natural teeth which are bounded by PDL, a healthy dental implant exhibits no mobility.

The fiber orientation around a natural tooth attaches perpendicular to the long axis of the tooth. This structure offers reisistance during periodontal probing. This perpendicular fiber orientation, absent around dental implants. The fiber orientation around an implant is parallel to the long axis of the implant. During periodontal probing of the implant sulcus, the probe passes between the gingival fibers and advances until it encounters the bone crest. Hence, repeated probing offers little information about dental implant health, such probing is indicated only when signs of infection, such as exudate, swelling, bleeeing, or inflammation are present. Plastic probes should be used to measure the peri-implant sulcus. Since the probing depth is related to the surrounding gingival thickness and may range from 1-4mm.. Since these depths may be greater than for healthy natural teeth, comparing probing depths against baseline and subsequent examination data is useful. The mucosal seal around a dental implant is a less effective barrier to bacterial plaque biofilm than the attachment apparatus around a natural tooth. Additionally, the peri-implant mucosa is less vascular. Taken together, these two factors increase vulnerability to pathogenic insult. During professional cleaning, metal instruments should be used on natural teeth and not used to probe or scale dental implants because metal surfaces can scratch, contaminate the implant-abutment surfaceor strip away surface coating on a dental implant. Plastic Teflon, and gold plated hand scalers and curettes should be used. Titanium dental implants can be polished with a rubber cup and nonabrasive polishing paste. (Cochran)

Radiographic evaluation of alveolar bone around the implant shows crestal bone height. Assessed regularly for changes against a baseline radiograph and subsequent examinations, radiographic record is useful. During the first year following dental implant restoration, an average bone loss of 1-1.5mm is predicted. Every subsequent year, 0.2mm is considered within normal limits. (Fritz).

Following restoration, dental implants should be on a 3 month maintenance schedule to check not only the implant, but also the adjacent teeth. If all the examination paramters are stable after one year, 4-6 month maintenance can be implemented. (Bauman). In screw retained restorations, the prosthesis should be removed at least once a year to more easily asses the status of the peri-implant tissues, implant mobility and the fixture itself. If symptoms of infection or radiographic evidence of bone loss around the implant are present, the implant is judged as ailing or failing (Meffert).

References

Bränemark PI. Introduction to osseointegration.In: Bränemark PI, Zarb G, Albrektsson T, eds. *Tissue Integrated Prostheses: Osseointegration in Clinical Dentistry.* Chicago: Quintessence; 1995;11-76.

Factors affecting late implant bone

Chung DM, Oh TJ, Lee J, Misch CE, Wang HL. *Int J Oral Maxillofac Implants*. 2007 Jan-Feb;22(1):117-26.

Albrektsson T, ZarbGA, Worthington P, ErikssonAR. The long-term efficacy of currently used dental implants: a review and proposed criteria of success. *Int J Oral Maxillofac Implants* 1986;1:11-25.

Comparative biology of chronic and aggressive periodontitis

Heitz-Mayfield LJ, Lang NP. *Periodontol* 2000. 2010 Jun;53:167-81.

Cochran, DL. Implant therapy I. *Ann Periodontol* 1996; 1:707-791.

Consensus Report: Implant therapy I. *Ann Periodontol* 1996;1:792-795.

Fritz, ME. Implant therapy II. *Ann Periodontol* 1996; 1:796-815.

Consensus report: Implant therapy II. *Ann Periodontol* 1996;816-818.

Bauman GR, Mills M, Rapley JW, Hallmon WH. Clinical parameters of evaluation during implant maintenance.*Int J Oral Maxillofac Implants* 1992;7:220-227.

Meffert RM. Treatment of failing dental implants. *Curr Opin Dent* 1992;2:109-114.

Imaging

CBCT is a method to acquire 3-dimensional radiographic images, which combines conventional x-ray and computerized volumetric reconstruction.It is digital by nature and uses a computer program to reconstruct a volume from 250-300 2-dimensional images.CBCT is becoming increasingly popular in dentistry because craniofacial CBCT was designed to counter some of the limitations of conventional, helical CT scanning devices.The radiation source consists of a conventional low-radiation x-ray tube.The beam is projected on a panel detector, producing a focused beam with less scatter radiation versus helical CT devices. The total radiation is significantly less than that of helical CT and is nearly equal to the exposure during a full-mouth series of dental x-rays. The image files are the DICOM (Digital Imaging and Communications in Medicine) format, the standard format for 3-dimensional images in medicine. Since all images can be taken in less than a minute, using a single rotation of the x-ray source, CBCT is useful in trauma, intraoperative, and sedation cases. (Palomo)

CBCT is smaller and less expensive than traditional CT and is particularly suited to evaluating the jaws because of a lower level of metal artifacts in reconstructions versus its helical predecessor.In a conventional CT, for instance, evaluation of an area of the jaws close to a metallic restoration, a crown or an implant is very difficult to analyze because of the artifacts and distortions created by metal Figure 35. Effective CBCT radiation dose depends on the settings used (kVp and mA), even though, overall, CBCT provides 3-dimensional volumetric images with up to 4 times less radiation than conventional CT.Using lower mA and / collimation reduce the amount of radiation but also adversely affect image quality.Exposure dose from CBCT is reported to be as low as 45MSv (Micro sieverts) to as high as 650 MSVExposure from a full mouth series of analog radiographs and panorex, as a reference is 150 MSv and 54 MSv respectively. (Palomo)

CBCT images, unlike conventional dental radiography,clearly identify buccolingual alveolar ridge deficiency. Conventional CT scans havebeen used to assess the osseous dimensions, relative bone density, cortical platethickness, and alveolar ridge height, and space between landmarks. (Palomo)Figure 36.

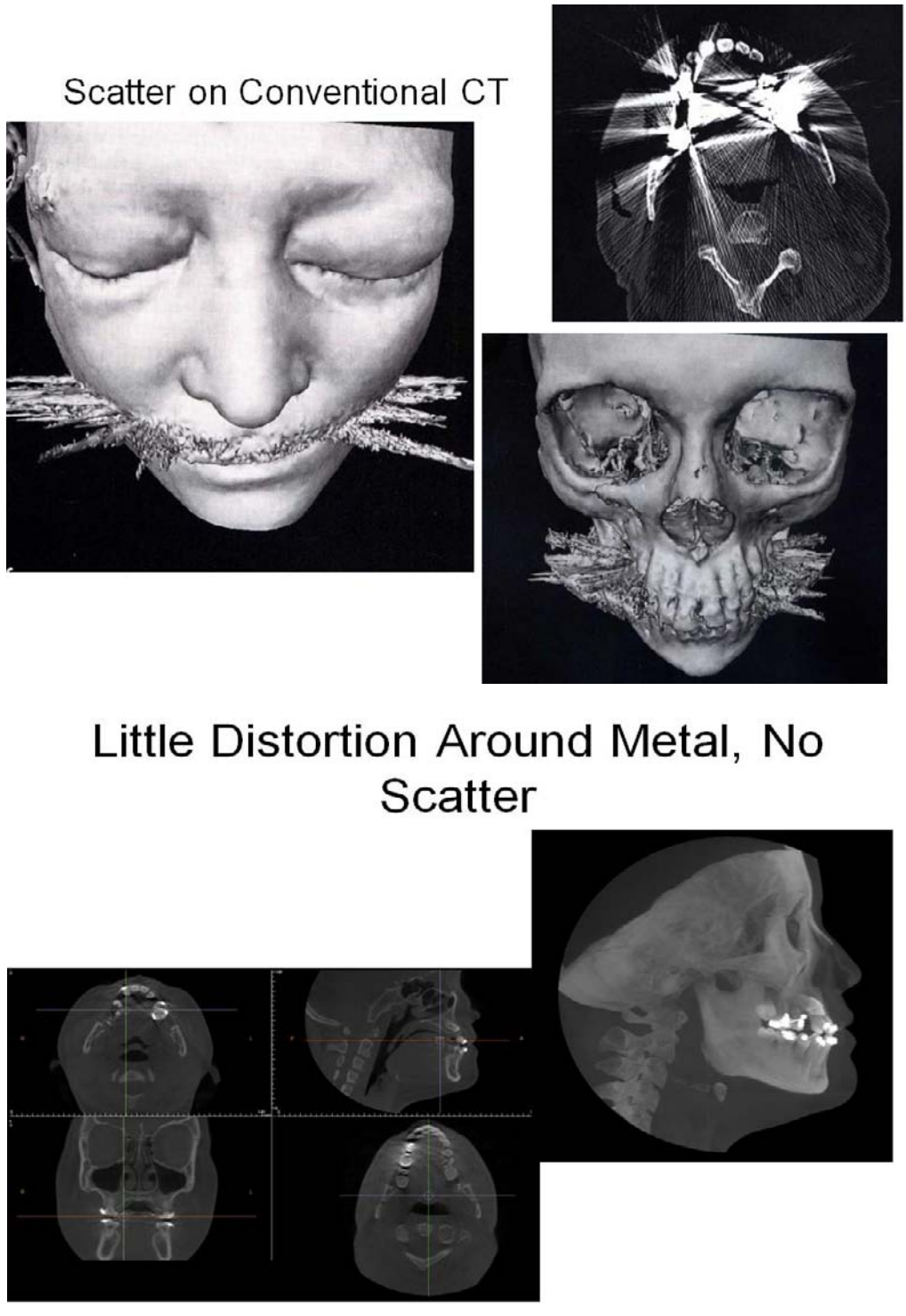

Scatter on Conventional CT
Little Distortion Around Metal, No Scatter

Figure 35.

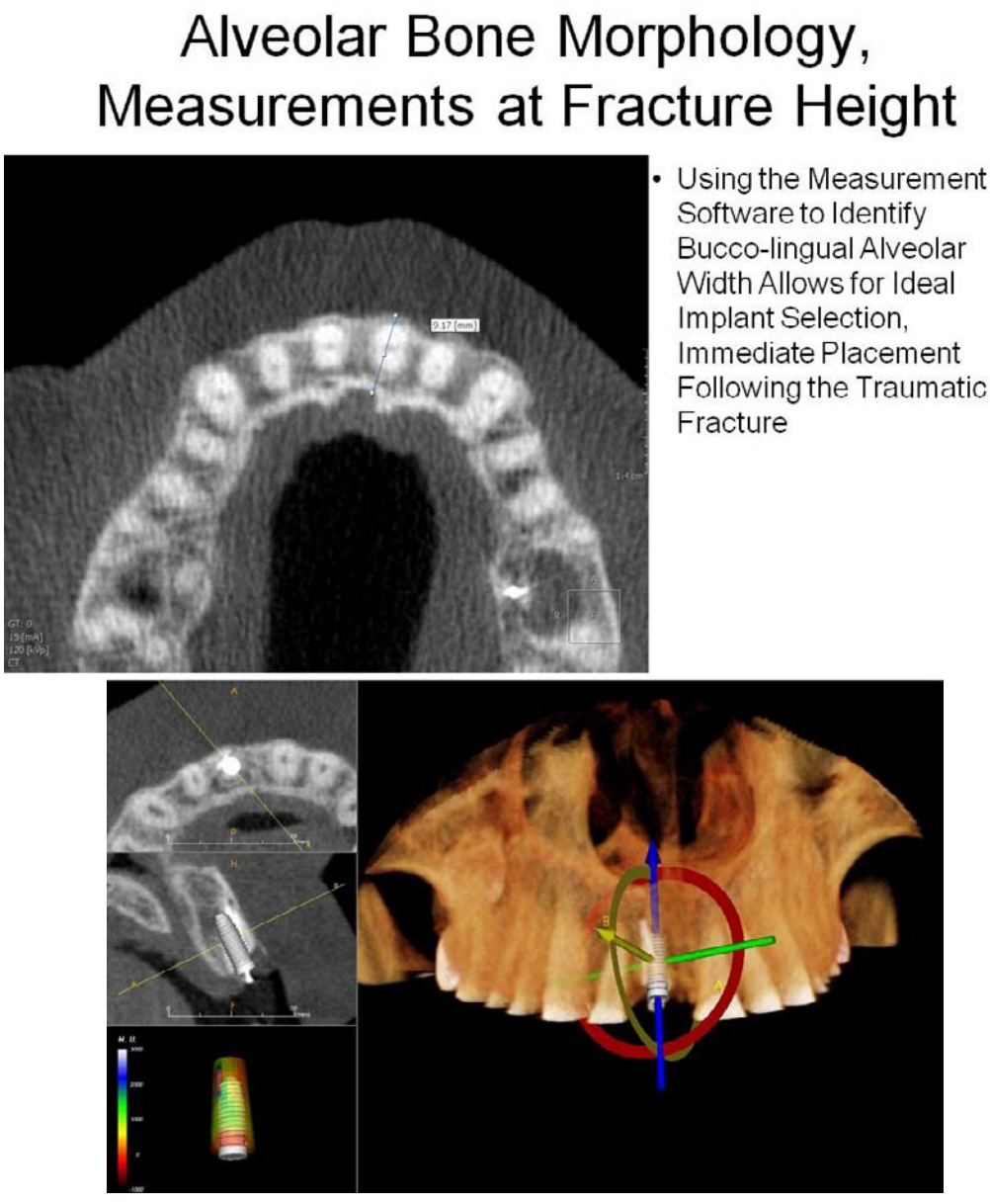


Figure 36.

Because of these views are available, CBCT images can be used to facilitate dental implant planning and placement. Many planning software products accommodate selection ofbrand-name implants and allow for selection of placement location and angulationsuch that available bone is used and local anatomy, such as adjacent teeth, nerves,and sinuses, are avoided. Laboratory-fabricated stereolithographic guides are useful for transferringthe planned surgery to the patient. This way, imaging helps virtually plan locations and angulations

References

Cone beam computed tomographyOsorio F, Perilla M, Doyle DJ, Palomo JM. *Anesth Analg.* 2008 Jun;106(6):1803-7.

Influence of CBCT exposure conditions on radiation dose. Palomo JM, Rao PS, Hans MG. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2008 Jun;105(6):773-82. Epub 2008 Apr 18.

Cone beam CT for diagnosis

Palomo L, Palomo JM. *Dent Clin North Am*. 2009 Oct;53(4):717-27, vi-vii.

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Chapter IX

Clinical Effects of 2% Chlorhexidine Gel on Patients Undergoing Orthodontic Treatment

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Abstract

Objectives: The purpose of this study was to compare the short-term clinical effects of a single intrasulcular injection of 2% chlorhexidine gluconate gel (CG) and placebo gel (PG) in orthodontic patients with fixed appliances and established gingivitis aged from 12 to 20 years.

Methods and Materials: 50 patients (31 females, 19 males) were divided into two groups (CG and PG) of 50 subjects. This study was single blind randomized split mouth clinical trial. As randomly assigned by coin toss, the first permanent molars on the right or left side of the mouth received either CG or PG. Probing depth (PD) was measured with a Michigan 0 probe. The gingival index (GI) of Löe and SILNESS and papilla bleeding index (PBI) of MÜHLEMANN were recorded on the first permanent molars. These indices were measured at baseline, and in treatment on second, fourth, eighth, and the twelfth weeks. T-test and chi-square test were used to analyze the data.

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Results: T-test showed that PD was reduced in experimental group in comparison with the control group in the 4th week and following intervals ($p<0.001$). Chi-square showed that PBI was improved in experimental group in comparison with the control group in the 2nd week and following intervals ($p<0.001$). The same test showed that GI was improved in experimental group in the 2nd week and following intervals ($p<0.001$).

Conclusion: The data indicate that the use of a single application of 2% CG was effective in reducing gingivitis related to banded first permanent premolars in adolescents undergoing orthodontic treatment in short time.

Keywords:*Chlorhexidine gel, gingival bleeding, periodontal pocket depth, orthodontics.*

Introduction

The presence of plaque is a key factor in most forms of periodontal disease [1]. Orthodontic appliances prevent removal of plaque by brushing, mastication and salivary flow. It is difficult to maintain good oral hygiene when orthodontic bands, wires and ligatures are placed [2]. The development of gingivitis within 1 to 2 months after placement of fixed orthodontic appliances has been reported [3]. Some authors have also noted slight attachment loss 2 years after removal of fixed orthodontic appliances when patients have not been continually motivated regarding oral hygiene [4,5]. Pockets of less than 3mm in depth can be maintained plaque-free by home care; Moreover, regular professional nonsurgical periodontal therapy can maintain the stability of deeper pockets [6,7].

Antibiotics and antiseptics have been used successfully to treat moderate-to-severe periodontal diseases [8-16]. It appears that the local application of antimicrobials that are effective against periodontopathogenes can reduce periodontal pocket depths [17]. Controlled-release antimicrobial delivery systems have also been tested as monotherapies, independent of scaling and root planning [12,14,18-21] (SRP) or in combination with mechanical debridement as adjunctive therapies [9-11,13,15,19,22-25].

Chlorhexidine is an effective antiplaque agent [26] although a drawback of chlorhexidine is pigmentation of teeth and oral tissues [27,28] Some clinical trials have shown that application of chlorhexidine has not significantly improved periodontal parameters [29,30], but PD has been significantly reduced by subgingival irrigation with 1% CG [31-33], which may have a role in the management of persistent pockets during chronic periodontitis. The effects of a single intrasulcular injection of 2%CG on the treatment of gingivitis in orthodontic patients which wearing fixed appliances have not been evaluated.

The purpose of this single blind placebo-controlled randomized clinical trial was to examine the effectiveness of single intrasulcular injection of 2% chlorhexidine gluconate gel on orthodontic patients with fixed orthodontic appliances and established gingivitis from 12 to 20 years.

Materials and Methods

Patients aged between 12 and 20 years receiving fixed appliance orthodontic treatment in a private practice were invited to take part in this trial. The trial was a single blind placebo controlled randomized split mouth clinical trial.The subjects were randomly assigned by coin toss, the first permanent molars on the right or left side of the mouth received either CG or PG. The subjects had not received any medication or periodontal treatment in the previous year and had no systemic complicating factors. 52 subjects gave informed written consent but two subjects subsequently withdrew from the trial due to personal reasons. To achieve a study with 95 per cent power of detecting a significanteffect with an alphalevel of 0.05, fifty patients were included in the sample size of thisstudy. The patients were undergoing either upper or lower fixed appliance therapy with a 018 standard edgewise system with brackets on the anterior teeth and bands on the first permanent molars. They were in treatment for more than 1 year. Each patient had existing gingivitis related to the first banded permanent molars on the basis of the following criteria.

1. A probing depth (PD) of 3mm or greater
2. GI (GI) (Löe and Silness) [34] grade 2 or greater
3. PBI (PBI) (Mühlemann) [35] grade 2 or greater.

PD was measured at six sites (Mesiobuccal, Midbuccal, Distobuccal, Mesiolingual, Midlingual and Distolingual) from the bottom of the pocket of the first banded permanent molar to the gingival margin with a Michigan 0 probe. GI was evaluated as indicated by Löe and Silness [34] at the mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual aspects of each first banded permanent molar. PBI was taken as indicated by Mühlemann [35] at the interdental papilla (mesial and distal) on each first banded permanent molar.

At baseline, either the two upper or two lower first permanent molars of each patient were randomized by a coin toss so that one molar was allocated to receive CG and the other PG. All periodontal recordings were taken by one single calibrated examiner. In the treatment group, moisture isolation was maintained by cotton wool rolls and air drying, prior to insertion of 2%CG (Consepsis® Scrub, Ultradent, Utah, USA) into the periodontal pocket. The gel was applied by a non-traumatic needle with a diameter of 0.65mm until the gingival sulcus was overfilled, and the periodontal pocket of the control first permanent molar was treated with a placebo (4% W/W methylcellulose 400 c P; 12.75% W/W glycerol; 0.2% W/W methylparahydroxybenzonate and 0.006% V/W peppermint oil). No other periodontal treatment such as scaling and root planing was performed. The reason that 2% CG was applied is that (Consepsis® Scrub, Ultradent, Utah, USA) factory only provided 2% CG injections at the moment of research.

PD, GI, and PBI, were measured at baseline and at the second, fourth, eight and twelfth weeks thereafter. All assessments were made by one single examiner. One single injection was applied at the base line and measurements were taken in the mentioned intervals.PBI and GI were divided into two groups from the2nd week interval. This division was done in order to simplify the comparison between control and experimental group; otherwise, more subdivisions had to be applied since (Löe and Silness) [34] and (Mühlemann) [35] have four

and five subdivisions respectively. In this division PBI and GI less than grade 2 was considered as one group and PBI and GI more than grade 2 was considered as another group.

Patients had previously received oral hygiene instructions prior to banding and bonding, and they were instructed to continue their usual oral hygiene procedures throughout the study. No additional attempts were made to improve the patients' oral hygiene. T-Test was used to measure PD in both experimental and control groups and Chi-square test was used to evaluate PBI and GI in both groups.

Results

T-test showed that PD in experimental group was 0.2 mm greater than control group in the baseline; however, this difference was not statistically significant. In the 2nd week the difference was not significant either. Nevertheless, PD in experimental group was 1.2 mm less than control group in the 4th week; moreover, this difference was statistically significant. PD in experimental group was 1.4 mm and 0.9 mm less than control group in 8th And 12th weeks intervals respectively; furthermore this difference was statistically significant (Table 1).

The number of patients based on PBI is shown in Table 2 in two groups. PBI was the same in both groups at the baseline; however, it was decreased in experimental group in the 2nd week interval in a way that PBI in experimental group was 64% less than control group. These differences continued in the following intervals and the differences between two groups were statistically significant from 2nd week until 12th week interval.

The number of patients based on GI is shown in Table 3 in two groups. GI was the same in both groups at the baseline; however, GI was improved in experimental group in comparison with control group in the 2nd week interval in a way that in this interval 36 patients of experimental group had GI less than grade 2, which was twice than control group; Moreover, this difference reached 19 times in the 12th week interval and the differences between two groups were statistically significant from 2nd week until 12th week interval.

Table 1. Periodontal Depth at the base line and at 2, 4, 8 and 12 weeks intervals in two groups

	Experimental group n = 50	Control group n = 50	P value
Base line	4.4+0.73	3.91+0.97	0.2
2nd week	4.06+0.38	3.87+0.6	0.06
4th week	2.27+0.46	3.56+0.45	0.001
8th week	2.39+0.54	3.77+0.43	0.001
12th week	2.44+0.51	3.37+0.33	0.001

Table 2. Number of samples based on PBI at the base line and at 2, 4, 8 and 12 weeks intervals in two groups

	Experimental group n = 50		Control group n = 50		P value
	< 2	2 >	< 2	2 >	
Base line	----	50(100)	----	50(100)	------
2nd week	43(86)	7(14)	11(22)	39(78)	0.001
4th week	38(76)	12(24)	7(14)	43(86)	0.001
8th week	41(82)	9(18)	5(10)	45(90)	0.001
12th week	40(80)	10(20)	3(16)	47(84)	0.001

Table 3. Number of samples based on GI at the base line and at 2, 4, 8 and 12 weeks intervals in two groups

	Experimental group n = 50		Control group n = 50		P value
	< 2	2 >	< 2	2 >	
Base line	----	50(100)	----	50(100)	------
2nd week	36(72)	14(28)	18(36)	32(64)	0.001
4th week	34(68)	16(32)	12(24)	38(76)	0.001
8th week	38(76)	12(24)	5(10)	45(90)	0.001
12th week	38(76)	12(24)	2(4)	48(96)	0.001

Discussion

The results of this study indicate that a single intrasulcular injection of 2% CG at the first visit was clinically effective at improving gingival health over a 3-month period in patients, aged between 12 to 20 years who were undergoing fixed appliance orthodontic therapy. The differences in the GI, PBI and probing depth using experimental and placebo groups indicate that application of 2% CG effectively reduces gingival inflammation, gingival bleeding and probing depth.

A single blind was used so that the examiner, and not the patient, was aware whether CG or PG had been used in relation to any molar tooth. A double-blind design would have been possible but was not undertaken.

In management of periodontal disease, a core element of therapy is effective tooth brushing. In some circumstances, however, chlorhexidine may be used as an adjunctive treatment. A possibility for those orthodontic patients who are undergoing a long treatment is the local application of CG by injection.

The results of the present study seem to agree with the findings of previous studies where chlorhexidine gluconate was used in a similar population [31,36,37]. Segreto *et al.* [38]found an average of 28% less gingival occurrence in a 3 month study. Grossman *et al.*[39] found that the decrease in gingival occurrence averaged 29% after 3 months and 37% after 6 months.The efficacy of chlorhexidine at a 1% concentration in treatment of chronic periodontitis has been established [12,40]. Unsal *et al.*[12] have reported that clinical and microbiological benefits can be achieved when subgingival administration of CG is an adjunct to scaling and root planing (SRP), as compared to SRP alone. Similar findings were reported by Fine *et al.*[40] regarding subgingival irrigation of chlorhexidine. Piccolomini *et al.*[41] have reported clinical and microbiological benefits after subgingival administration of CG as an alternative to SRP.Lorenz *et al.*[42] found that the new Chlorhexidine mouthrinses were able to inhibit plaque re-growth and gingivitis.

Subgingival access for antimicrobial irrigation is important[43,44] and oral irrigating devices have been shown to be useful for the delivery of antimicrobial agents in effective dosages [45,46]. It is possible that the mechanism for reduction of the gingival inflammation associated with subgingival delivery may be due to the reduction of specific microorganisms or toxic products of plaque[47] or by a disruption of subgingival plaque rather than instant killing of microorganisms [48].

In a longitudinal clinical study on the gingival condition of young patients (aged 11 to 13 years) treated with fixed orthodontic appliances, it was determined that despite repeated motivation in tooth brushing technique and sodium fluoride rinses twice weekly, most of the children developed generalized gingivitis within 1 to 2 months after placement of appliances [3]. Follow up studies [4,5] reported attachment loss in the orthodontically treated group. It is important to notice that the subjects had not been continually motivated with respect to their oral hygiene habits. A thorough home care program that includes direct involvement with the child's parents seems to be in order and has been suggested [49].

Diamanti-Kipioti *et al.*[50] reported that placement of orthodontic bands in children will favor pseudo pocket formation. The use of chlorhexidine may be used as a motivating factor for patients, as Ainamo[51] has suggested. It would make the patients aware of the sensation of cleanliness, and this awareness will increase patients' ability of removing plaque.

One of the side effects of chlorhexidine in the form of a mouth rinse is staining, which may be of aesthetic concern to the patient. In this study no staining was observed on any of the patients' teeth. This may be because chlorhexidine in gel form was injected locally in the subgingiva rather than administering a mouth rinse.

The taste of chlorhexidine may be bitter, but its advantages outweigh the disadvantages and make it an acceptable chemical agent for reducing plaque and gingivitis. The concept of a chemical agent to enhance oral health has long been considered and the importance of such an agent is even greater in orthodontic patients with established gingivitis. Chlorhexidine is an important therapeutic agent in controlling gingival inflammation due to its antimicrobial activity [26,47,48].

Orthodontic patients wearing fixed appliances where gingivitis is evident can benefit from CG injection. It is recommended that orthodontists apply a single intrasulcular injection of 2% CG in such circumstances, aside from scaling and root planing.

Conclusion

- The single intrasulcular injection of 2% CG is an effective procedure in controlling gingival inflammation in adolescents undergoing fixed orthodontic treatment in short time.
- Single application of 2% CG does not ensure that all subgingival sites are disease free.

References

[1] Lindhe J. Textbook of Clinical Periodontology, 2nd ed. Copenhagen: Munksgaard; 1989.

[2] Lundström F, Hamp SE. Effect of oral hygiene education on children with and without subsequent orthodontic treatment. *Scand. J. Dent. Res.* 1980; 88:53-9.

[3] Zachrisson S, Zachrisson BU. Gingival condition associated with orthodontic treatment. *Angle Orthod.* 1972; 42:26-34.

[4] Zachrisson BU, Alnaes L. Periodontal condition in orthodontically treated and untreated individuals. I. Loss of attachment, gingival pocket depth and clinical crown height. *Angle Orthod.* 1973; 43:402-11.

[5] Zachrisson Bu, Alnaes L.Periodontal condition in orthodontically treated and untreated individuals. II. Alveolar bone loss: radiographic findings. *Angle Orthod*. 1974; 44:48-55.

[6] Berkey CS, Antczak-Bouckoms A, Hoaglin DC, Mosteller F, Pihlstrom BL. Multiple-outcomes meta-analysis of treatments for periodontal disease. *J. Dent. Res*. 1995; 74:1030-9.

[7] Kaldahl WB, Kalkwarf KL, Patil KD, Molvar MP, Dyer JK. Long-term evaluation of periodontal therapy: I. Response to 4 therapeutic modalities. *J. Periodontol.* 1996; 67:93-102.

[8] Rams TE, Slots J. Local delivery of antimicrobial agents in the periodontal pocket. . *J. Clin. Periodontol.* 1996; 10:139-59.

[9] Noyan U, Yilmaz S, Kuru B, Kadir T, Acar O, Buget E. A clinical and microbiological evaluation of systemic and local metronidazole delivery in adult periodontitis patients. *J. Clin. Periodontol.* 1997; 24:158-65.

[10] Palmer RM, Matthews JP, Wilson RF. Adjunctive systemic and locally delivered metronidazole in the treatment of periodontitis: a controlled clinical study. *Br. Dent. J.* 1998; 184:548-52.

[11] Unsal E, Akkaya M, Walsh TF. Influence of a single application of subgingival chlorhexidine gel or tetracycline paste on the clinical parameters of adult periodontitis patients. *J. Clin. Periodontol*. 1994; 21:351-5.

[12] Unsal E, Walsh TF, Akkaya M. The effect of a single application of subgingival antimicrobial or mechanical therapy on the clinical parameters of juvenile periodontitis. *J. Periodontol*. 1995; 66:47-51.

[13] Lie T, Bruun G, Boe OE. Effects of topical metronidazole and tetracycline in treatment of adult periodontitis. *J. Periodontol.* 1998; 69:819-27.

[14] Knoll-Kohler E. Metronidazole dental gel as an alternative to scaling and root planing in the treatment of localized adult periodontitis. *Eur. J. Oral Sci.* 1999; 107:415-21.

[15] Griffiths GS, Smart GJ, Bulman JS, Weiss G, Shrowder J, Newman HN. Comparison of clinical outcomes following treatment of chronic adult periodontitis with subgingival scaling or subgingival scaling plus metronidazole gel. *J. Clin. Periodontol*. 2000; 27:910-7.

[16] Vinholis AH, Figueiredo LC, Marcantonio Junior E, Marcantonio RA, Salvador SL, Goissis G. Subgingival utilization of a 1% chlorhexidine collagen gel for the treatment of periodontal pockets. A clinical and microbiological study. *Braz. Dent. J.* 2001; 12:209-13.

[17] Okuda K, Wolff L, Oliver R, Osborn J, Stoltenberg J, Bereuter J, Anderson L, Foster P, Hardie N, Aeppli D, et al. Minocycline slow-release formulation effect on subgingival bacteria. *J. Periodontol.* 1992; 63:73-9.

[18] Rudhart A, Purucker P, Kage A, Hopfenmuller W, Bernimoulin JP. Local metronidazole application in maintenance patients. Clinical and microbiological evaluation. *J. Periodontol.* 1998; 69:1148-54.

[19] Awartani FA, Zulqarnain BJ. Comparison of the clinical effects of subgingival application of metronidazole 25% gel and scaling in the treatment of adult periodontitis. *Quintessence Int*. 1998; 29:41-8.

[20] Stelzel M, Flores-de-Jacoby L.Topical metronidazole application in recall patients. Long-term results. *J. Clin. Periodontol.* 1997; 24:914-9.

[21] Pedrazzoli V, Kilian M, Karring T. Comparative clinical and microbiological effects of topical subgingival application of metronidazole 25% dental gel and scaling in the treatment of adult periodontitis. *J. Clin. Periodontol*. 1992; 19:715-22.

[22] Vandekerckhove BN, Bollen CM, Dekeyser C, Darius P, Quirynen M. Full- versus partial-mouth disinfection in the treatment of periodontal infections. Long-term clinical observations of a pilot study. *J. Periodontol.* 1996; 67:1251-9.

[23] Kinane DF, Radvar M. A six-month comparison of three periodontal local antimicrobial therapies in persistent periodontal pockets. *J. Periodontol.* 1999; 70:1-7.

[24] Riep B, Purucker P, Bernimoulin JP. Repeated local metronidazole-therapy as adjunct to scaling and root planing in maintenance patients. *J. Clin. Periodontol*. 1999 ; 26:710-5.

[25] Stelzel M, Flores-de-Jacoby L. Topical metronidazole application as an adjunct to scaling and root planing. *J. Clin. Periodontol.* 2000; 27:447-52.

[26] [26] Löe H, Schiott CR. The effect of mouthrinses and topical application of chlorhexidine on the development of dental plaque and gingivitis in man. *J. Periodontal. Res.* 1970; 5:79-83

[27] Eriksen HM, Nordbo H, Kantanen H, Ellingsen JE. Chemical plaque control and extrinsic tooth discoloration. A review of possible mechanisms. *J. Clin. Periodontol.* 1985; 12:345-50.

[28] Addy M, Moran J, Davies RM, Beak A, Lewis A. The effect of single morning and evening rinses of chlorhexidine on the development of tooth staining and plaque accumulation. A blind cross-over trial. *J. Clin. Periodontol.* 1982; 9:134-40.

[29] Lundstrom F, Hamp SE, Nyman S. Systematic plaque control in children undergoing long-term orthodontic treatment. *Eur. J. Orthod.* 1980; 2:27-39.

[30] Morrow D, Wood DP, Speechley M. Clinical effect of subgingival chlorhexidine irrigation on gingivitis in adolescent orthodontic patients. *Am. J. Orthod. Dentofacial Orthop.* 1992; 101:408-13.

[31] Brightman LJ, Terezhalmy GT, Greenwell H, Jacobs M, Enlow DH. The effects of a 0.12% chlorhexidine gluconate mouthrinse on orthodontic patients aged 11 through 17 with established gingivitis. *Am. J. Orthod. Dentofacial Orthop*. 1991; 100:324-9.

[32] Perinetti G, Paolantonio M, Cordella C, D'Ercole S, Serra E, Piccolomini R. Clinical and microbiological effects of subgingival administration of two active gels on persistent pockets of chronic periodontitis patients. *J. Clin. Periodontol.* 2004; 31:273-81.

[33] Babay N, Al Jasser N. Subgingival irrigation effects of chlorhexidine or sanguinarine on gingivitis in orthodontic patients. *J. Clin. Pediatr. Dent.* 1996; 20:225-8.

[34] James D,Beck J, Samuel J,Arbes J. Epidemiology of Gingival and Periodontal Diseases.In:Newman MG,Takei HH,Carranza FA.Carranza's clinical periodontology. Page: 80. 9th ed.Philadelphia: WB Saundaers; 2000.

[35] Saxer UP, Muhlemann HR. Motivation and Education. SSO Schweiz Monatsschr Zahnheilkd 1975; 85:905-19.

[36] Lang NP, Hotz P, Graf H, Geering AH, Saxer UP, Sturzenberger OP, Meckel AH. Effects of supervised chlorhexidine mouthrinses in children. A longitudinal clinical trial. *J. Periodontal Res.* 1982; 17:101-11.

[37] Anderson GB, Bowden J, Morrison EC, Caffesse RG. Clinical effects of chlorhexidine mouthwashes on patients undergoing orthodontic treatment. *Am. J. Orthod. Dentofacial Orthop*. 1997; 111:606-12.

[38] Segreto VA, Collins EM, Beiswanger BB, et al. A comparison of mouthrinses containing two concentrations of chlorhexidine. *J. Period. Res.* 1986:23-32.

[39] Grossman E, Reiter D, Sturzenberger OP, et al. Six month study of the effects of a chlorhexidine mouthrinse on gingivits in adults. *J. Periodont. Res*. 1986; 16:33-43.

[40] Fine JB, Harper DS, Gordon JM, Hovliaras CA, Charles CH. Short-term microbiological and clinical effects of subgingival irrigation with an antimicrobial mouthrinse. *J. Periodontol.* 1994; 65:30-6.

[41] Piccolomini R, Di Bonaventura G, Catamo G, Tumini V, Di Placido G, D'Ercole S, Perfetti G, Paolantonio M. Microbiological and clinical effects of a 1% chlorhexidine-gel in untreated periodontal pockets from adult periodontitis patients. New Microbiol 1999; 22:111-6.

[42] Lorenz K, Bruhn G, Heumann C, Netuschil L, Brecx M, Hoffmann T. Effect of two new chlorhexidine mouthrinses on the development of dental plaque, gingivitis, and discolouration. A randomized, investigator-blind, placebo-controlled, 3-week experimental gingivitis study. *J. Clin. Periodontol.* 2006; 33:561-7.

[43] Pitcher GR, Newman HN, Strahan JD. Access to subgingival plaque by disclosing agents using mouthrinsing and direct irrigation. *J. Clin. Periodontol.* 1980; 7:300-8.

[44] Eakle WS, Ford C, Boyd RL. Depth of penetration in periodontal pockets with oral irrigation. *J. Clin. Periodontol*. 1986; 13:39-44.

[45] [45] Lang NP, Raber K. Use of oral irrigators as vehicle for the application of antimicrobial agents in chemical plaque control. *J. Clin. Periodontol*. 1981; 8:177-88.

[46] Lang NP, Ramseier-Grossmann K. Optimal dosage of chlorhexidine digluconate in chemical plaque control when applied by the oral irrigator. *J. Clin. Periodontol.* 1981; 8:189-202.

[47] Flemmig TF, Newman MG, Doherty FM, Grossman E, Meckel AH, Bakdash MB. Supragingival irrigation with 0.06% chlorhexidine in naturally occurring gingivitis. I. 6 month clinical observations. *J. Periodontol*. 1990; 61:112-7.

[48] Schlagenhauf U, Stellwag P, Fiedler A. Subgingival irrigation in the maintenance phase of periodontal therapy. *J. Clin. Periodontol.* 1990; 17:650-3.

[49] Kloehn JS, Pfeifer JS. The effect of orthodontic treatment on the periodontium. *Angle Orthod.* 1974; 44:127-34.

[50] Diamanti-Kipioti A, Gusberti FA, Lang NP. Clinical and microbiological effects of fixed orthodontic appliances. *J. Clin. Periodontol.* 1987; 14:326-33.

[51] Ainamo J. Control of plaque by chemical agents. *J. Clin. Periodontol*. 1977; 4:23-35.

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Chapter X

Periodontal Disease and Systemic Diseases: Interrelationships and Interactions

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Abstract

The focal infection theory, which for almost half a century justified indiscriminate extraction of teeth to cure focal infections, since the end of the 1940s has become progressively a discarded concept. In parallel with the declining importance assigned to pulp and periapical infections in the pathogenesis of focal diseases, over the last decade there has been increasing interest in the possible relationship between periodontal infection and systemic diseases. Periodontal pathogens and their products, as well as inflammatory mediators produced in gingival tissue, might enter the bloodstream through ulcerated pocket epithelium, causing systemic effects (focal diseases).

On the basis of this mechanism, chronic periodontitis has been implicated as risk factor for cardiovascular diseases associated to atherosclerosis, bacterial endocarditis, diabetes mellitus, respiratory disease preterm delivery, rheumatoid arthritis, and more recently osteoporosis, pancreatic cancer, metabolic syndrome, renal diseases and neurodegenerative diseases such as Alzheimer's disease. Numerous hypotheses, including common susceptibility, systemic inflammation, direct bacterial infection and cross-reactivity, or molecular mimicry, between bacterial antigens and self-antigens, have been postulated to explain these relationships. In this context, the association of periodontal disease with systemic diseases has introduced the concept of "periodontal medicine", which ultimately guides the medical community in therapeutic approaches to improve not only the patient oral health but also systemic health.

This chapter summarizes the pathophysiology of periodontal disease and presentsan update on interrelationships and interactions between periodontal disease and systemic

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diseases. Moreover, this chapter reviews the published literature that describes the effects of periodontal treatment on cardiovascular diseases, adverse pregnancy outcomes, diabetes mellitus, and respiratory disease.

Introduction

Oral conditions such as caries, gingivitis and chronic periodontitis are among the most prevalent microbial diseases. The cause of these common inflammatory conditions is the complex microbial biofilm, so called dental plaque. These microorganisms and their metabolic products, as well as inflammatory mediators, might enter the bloodstream, during mastication or therapeutical procedures, through ulcerated pocket epithelium, causing systemic effects (focal diseases).Focal oral infections can be defined as infections occurring in different sites of the human body and caused by microorganisms or their products that colonized the oral cavity.

Oral infections have been considered as a source of "focal infections" for a long time. The concept of "focal infection" from the beginning of the twentieth century is gaining ground in its new apparition and a substantial number of articles have been published to elucidate the relationships between oral and systemic diseases. The reawakening interest in the focal infection theory, and particularly, the association of chronic periodontitis with general medicine have leading to introduce the concept of "periodontal medicine" that ultimately guide the medical class in therapeutic and decisional approaches to improve not only the patient oral health but also the systemic health.

This section of book summarized the history of the focal infection theory and the pathophysiology of periodontal diseases. It also presents an update on interrelationships and interactions between chronic periodontitis and systemic diseases. The effects of periodontal treatment on systemic disease are also discussed.

The Theory of Focal Infection: Historical Perspective

In 1891 W. Miller published an article entitled "The Human Mouth as a Focus of Infection" suggesting the role of oral microorganisms or their products in the development of a variety of diseases at sites distant from the oral cavity, including brain abscesses, pulmonary diseases and gastric problems, as well as a number of systemic infectious diseases [1]. The theory of focal infection was revised during the 19^{th} and 20^{th} centuries. In 1912, F. Billings introduced the term focal infection and hypothesized that infected teeth and tonsils were responsible for arthritis, rheumatism, nephritis, appendicitis, endocarditis, and other unexplained diseases [2].Billings guides the activities of some researchers who study various cases of patients suffering from arthritis and nephritis, which improved after surgical removal of suspected foci of infection [3]. These findings were supported by the work of E.C. Rosenow, with whom Billings collaborated extensivelyand the focal infection theory gained wide acceptance among medical and dental professionals [4, 5]. Proponents of this concept assumed that microorganisms of the dental plaque and those

responsible of dental caries, together with their metabolic products, may enter in the blood torrent during mastication or therapeutical procedures and result in numerous and sometimes degenerative systemic conditions [6-8].

Originally, endodontically treated teeth were implicated as the main focus of infection, and in this context, is been suggested that some bacteria, such as *Streptococcus mutans* and *S.sobrinus*, *Lactobacilli* spp, *Actinomyces* spp and *Enterococcus faecalis*[9], remained in dentinal tubules following root canal therapy and produced toxins which are able to be disseminated through the cementum and root canal sealers into the surrounding periodontium and bloodstream. Price reported that several diseases was alleviated by dental extraction and many dental professionals interpreted these results as a recommendation to eradicate many chronic degenerative diseases by extracting diseased teeth, so therapeutic edentulism was thought as preventive factor respect to the focal infection theory [6, 7]. However, Cecil and Angevine, two early proponents of the theory, reversed their opinions when a 1938 study on rheumatoid arthritis patients showed that over 70% of these individuals had no evidence of focal infections [10].

In the 1940s a re-examination of these studies, progresses in radiology, and endodontic success rates led to the downfall of the focal infection theory, and so for the next several decades, the theory was discredited and largely ignored by medical community. Finally, the British Dental Association and the American Association of Endodontists have taken official positions disproving any potential connection between endodontic lesions and subsequent systemic health events, so the teeth treated endodontically not seem to be responsible for systemic disease [11, 12]. On the contrary the concept of focal infection have progressively gained credibility and with regard to the correlation between chronic periodontitis (CP) and systemic diseases.

On the basis of this new concept, in the last decade, CP has been implicated as risk factor for a number of systemic diseases including cardiovascular diseases associated to atherosclerosis, bacterial endocarditis, diabetes mellitus, respiratory disease, preterm delivery, rheumatoid arthritis, and more recently osteoporosis, pancreatic cancer, metabolic syndrome, kidney diseases and neurodegenerative diseases such as Alzheimer's disease.

In this context, in 1996 World Workshop in Periodontics, Offenbacher introduced the term, "periodontal medicine" as a discipline that focuses on validating of this relationship and its biological plausibility in human populations and animal models [13]. In July 1998, the American Academy of Periodontology launched an effort to educate the public about new findings which support what dentist had long suspected: "infections in the mouth can play an important role in disorders affected another sites in the body". For a long time it was thought that bacteria was the factor that linked CP to other infections in the body, whereas successfully new research demonstrates that not only the bacteria but also the periodontal inflammation may link periodontitis to other chronic conditions. The pioneering approach of "periodontal medicine" have permit a renovate attention of the "focal infection theory", deepening the relationship between periodontitis and systemic health.

Pathogenic Mechanisms Linking Oral Infection and Systemic Diseases

Dental procedures, including tooth extraction, endodontic treatment, periodontal surgery, scaling and root planning can provoke the introduction of oral microorganisms into the bloodstream[14]. CP can also favors the access of bacteria into connective tissues and the bloodstream. Similarly, chewing and oral hygiene procedures can induce spontaneous bacteremia, more particularly if the subjects present poor oral health[15]. It is thus evident that the risk of developing focal infections from bacteremia resulting from dental treatment is far lower than previously thought and that the cumulative exposure to bacteremia is significantly greater from everyday procedures when compared to dental operative procedures. For instance, a report from Guntheroth [16] revealed that the cumulative monthly bacteremic exposure resulting from self-induced oral procedures (chewing and tooth brushing) is about 1,000 times greater than from a dental extraction.

Generally, three different etiological mechanisms by which oral bacteria may cause systemic diseases have been proposed:

1. metastatic infection caused by translocation of Gram negative bacteria that gain access to the blood torrent as a result of breach of the compromised epithelial lining of periodontal pockets;
2. metastatic injury related to the effects of the circulating exo-toxins (e.g. cytolitic enzymes) and endo-toxins (e.g. lipopolysaccharide (LPS) of periodontal pathogens;
3. metastatic inflammation due to the immunologic response to the pathogens and their toxins. Soluble antigens may enter the bloodstream, react with circulating specific antibody, and shaped a macromolecular complex. These immunocomplexes may give rise to a variety of acute and chronic inflammatory reactions at the sites of deposition[17, 18].

Possible pathways of oral infections and systemic diseases are showed in Table 1.

Table 1. Possible pathways between oral infections and systemic diseases

Pathway for oral infection	Increased risk for
Metastatic infection caused by translocation of Gram negative bacteria	Acute bacterial myocarditis, subacute infective endocarditis, brain abscess, cavernous sinus thrombosis, sinusitis, lung abscess/infection, orbital cellulitis, Ludwig's angina, skin ulcer, osteomyelitis, prosthetic joint infection.
Metastatic injury related to the effects of the circulating oral microbial toxins of periodontal pathogens	Cerebral infarction, acute myocardial infarction, adverse pregnancy, persistent pyrexia, idiopathic trigeminal neuralgia, toxic shock syndrome, systemic granulocytic cell defects, chronic meningitis.
Metastatic inflammation due to the immunologic response to the pathogens and their toxins	Behcet's syndrome, uveitis, chronic urticaria, inflammatory bowel disease, Crohn's disease.

Epidemiological studies have shown the association between chronic periodontitis and cardiovascular disease, respiratory diseases, diabetes, osteoporosis[19], preterm low birth weight[20]and, more recently, pancreatic cancer[21], metabolic syndrome[22], chronic kidney disease [23], rheumatoid arthritis[24]and neurodegenerative diseases such as Alzheimer's disease [25].

Pathophysiologyof Periodontal Diseases

Periodontal diseases include the infections affecting only the gingiva (i.e. gingivitis) and those affecting the tooth supporting tissues comprise periodontal ligament and the alveolar bone (i.e. periodontitis). Gingivitis is defined as the result of a non-specific inflammatory reaction in response to an increase in the mass of bacteria Gram-negative or Gram-positive, at/or under the gingival crevice. Conversely, periodontitis is initiated by an overgrowth of specific Gram-negative bacteria in the gingival crevice that lead to formation of a periodontal pocket which greatly favors further accumulation and a shift in the qualitative composition of bacteria. Clinically, gingivitisis characterized by erythematous and oedematous gingival tissue, which bleeds easily after periodontal probing and gentle brushing. Gingivitis is a reversible event and if treated with excellent oral hygiene the prognosis is good, but otherwise it may progress to periodontitis. Periodontitis is characterized by the periodontal ligament detachment from the cement, alveolar bone resorption, gingival recession, tooth migration, development of diastemas between the teeth loosening, mobility and at long last, abscess and tooth loss. The cause of these common inflammatory conditions is the dental plaque and it been demonstrate that one mg of this contain more than 10^{11} microorganisms and approximately 500 species[26].

The conversion from gingivitis to periodontitis, characterized by the initial loss of periodontal connective attachment and periodontal pocket formation, has been associated with the appearance of *Tannerella forsythia* (previously *Bacteroides forsythus*), *Campylobacter rectus* and *Selenomonas*spp. in the subgingival plaque. Furthermore, in adult populations with established periodontitis, sites with active phases of tissue destruction have been associated with *Aggregatibacter actinomycetemcomitans,* (previously*Actinobacillus actinomycetemcomitans),Eubacterium timidim,Fusobacterium nucleatum, Peptostreptococcus micros,Porphyromonas gingivalis, Prevotella intermedia* and *Treponema denticola*[27].

Some of these bacteria, and more particularly *F. nucleatum* and black-pigmented anaerobic bacteria, can also be found on other mucosal surfaces (e.g. gastrointestinal and genitourinary tracts) [28] and exist direct evidence on the role of some periodontal pathogensin atherosclerosis because the their identification in human atherosclerotic plaques carotid biopsies is been reported [29].

In a review article [18], Page proposed that periodontitis may affect the host's susceptibility to systemic disease in three ways:

1) by common risk factors: environmental risk factors (e.g. tobacco smoking, stress, aging, race or ethnicity, and gender) may place the individuals at high risk for periodontitis and at high risk for systemic diseases;

2) by subgingival biofilms: subgingival floraacting as reservoirs of Gram-negative bacteria,constituting an enormous and continuing bacterial cargo. They present continually renewing reservoirs of LPS and other Gram-negative bacteria with ready access to the periodontal tissues and the blood circulation. Furthermore, LPS induces major vascular responses, including an inflammatory cell infiltrate in the vessel walls, vascular smooth muscle proliferation, vascular fatty degeneration, and intravascular coagulation and up-regulates expression of endothelial cell adhesion molecules and secretion of interleukin-1 (IL-1), tumor necrosis factor alpha (TNF-a), and thromboxane, which results in platelet aggregation and adhesion, formation of lipid laden foam cells and deposits of cholesterol;
3) through the periodontium acting as a reservoir of inflammatory mediators, particularly of cytokine:the proinflammatory cytokines TNF-ά, IL-1β, and γ-interferon as well as prostaglandin E2 (PGE2) arrive at high tissue concentrations in CP.

The periodontium can therefore serve as a renewing reservoir for over flow of these mediators, which can enter in circulation and induce continue systemic effects.

In advanced stages of CP, the gingival tissue is markedly separate from the teeth. Continuing destruction of the periodontal attachment and deepening periodontal pockets can leading to significant loss of tooth-supporting tissues and alveolar bone. In these conditions, the thin, highly permeable, and frequently ulcerated pocket epithelium is the only barrier between the bacterial biofilm and the underlying connective tissues. Pocket epithelium is easily crossed by bacterial toxins and other products that accessed to the tooth supporting connective tissues and blood vessels.

It is evident that in the presence of severe periodontitis, microbial-induced infection presents a substantial infectious burden for the entire body by releasing bacteria, toxins, and other inflammatory mediators into the bloodstream that then affect other parts of the body. Still, the total burden of infection and inflammation will vary from one patient to the next, but could still represent a significant risk-factor. This notion represents a paradigm shift in thinking about the directionality of oral and systemic associations [30].

A number of hypotheses, including common susceptibility involving a genetically determined phenotype, direct bacterial damage to the endothelium, systemic inflammation with increased circulating cytokines and mediators and, finally, cross-reactivity or molecular mimicry between bacterial antigens and self-antigens, have been proposed to explain the relationship [31].

Periodontal Disease and Adverse Pregnancy Outcomes

Low birth weight (LBW) (< 2500 grams), and preterm birth (PTB) (before 37 weeks gestation) are the major determinant of neonatal infant morbidity and mortality with tremendous financial impact on public health systems[32].

These events have been treated as a single entity in most studies, although in other studies these have been unified and indicated with the acronym PLBW. A precise mechanism of

causes of PLBW cannot be established but there are many maternal or fetal characteristics that have been investigated, including maternal age (<18 or >40 years), nutritional deficit, previous PTB history, psychological aspect, inadequate prenatal care, diabetes mellitus,hypertension, excessive uterine contractions, premature cervical dilatation, adverse behaviors such as smoking, drug and alcohol abuse, genitourinary tract infection, cervical length, low socioeconomic status, biological and genetic factors. Since many of the risk factors result in increased systemic inflammation, the stimulation of infection or inflammation pathway might explain the increases in PLBW associated with multiple risk factors [32].There is a significant body of literature suggesting an important role of infection/inflammation in adverse pregnancy outcomes [33, 34] and a number of cases between 30 to 50% of PTB are thought to be caused by maternal infections.

Both PTB and LBW are possibly associated with periodontal disease[35]. The relationship between chronic periodontits and adverse pregnancy outcomes, in fact, is biologically plausible. The potential effect of periodontitis might be explained by several possible mechanisms [35]: translocation of periodontal pathogens to the fetoplacental unit, action of a periodontal reservoir of endotoxin (LPS) on the fetoplacental unit, or action of a periodontal reservoir of inflammatory mediators (interleukin-1ά [IL-1β], IL-6, tumor necrosis factor [TNF-ά] or prostaglandin E2 [PGE2]) on the fetoplacental unit, precipitating preterm labor by increasing the systemic circulation of cytokines. It is possible that the fetal-placental unit in women with progressive periodontitis is exposed to inflammatory mediators that precipitate pre-term labor and delivery. In fact, CP can cause an ulcerated lesion at the tooth-gingiva junction and the key periodontal pathogens, particularly *Porphyromonas gingivalis*, that are highly invasive in connective tissue, are capable of evading cellular immune defence mechanisms. Generally, periodontal bacteria activate cell-mediated immunological responses, leading to the production of pro-inflammatory cytokines, mainly IL-1β, IL-6, PGE2, and TNF-ά, matrix metalloproteinases and release of endotoxins such as lipopolysaccharides (LPS) or maternal inflammatory mediators, which may precipitate preterm labor if they arrive at the fetal-placental unit [36].

It has been established that mothers with PLBW had higher concentrations of four bacterial species typical of the periodontitis (*Tannerella forsythia*, *Porphyromonas gingivalis*, *Aggregaticbacter actinomycetemcomitans*, and *Treponemadenticola*), when compared to healthy controls [37], and that fetal IgM antibodies against periodontal pathogens, such as *P. gingivalis*, *Campylobacter rectus*, *Fusobacterium nucleatum*, have been reported in preterm infants than term infants (33.3% versus 17.9%) [38].

In this context, several studies showed a significant association between chronic periodontal disease and adverse pregnancy outcomes, including PTB [35, 39], preterm prelabour rupture of the membranes (and pre-eclampsia [40]. Three recent meta-analyses found a statistically significant association between chronic periodontitis and adverse pregnancy outcomes [41-43], even if recently Srinivas et al. [44] in a large prospective study failed to demonstrate this association. The nature and consistency of the association, therefore, continues to be debated.

Observational studies have reported on the necessity of treatment of women in pregnancy with periodontal disease for prevent the adverse pregnancy outcomes [35, 39]. Generally, the aims of treatment of periodontal disease are to allow resolution of the inflammation reducing the amount of plaque and calculus, and prevent or limit the tissue destruction to preserve

dentition. Periodontal treatment was not hazardous to the women or their pregnancies, but the treatment cannot be considered a preventing therapy for PLBW [45, 46].Although instruction and motivation of the woman to oral hygiene care are very important components of periodontal treatment, in the scenario of a link between chronic periodontitis and adverse pregnancy outcomes, a consensus has emerged, emphasizing the need for more studies on the effects of periodontal treatment in reducing the occurrence of PLBW.

Periodontal Disease and Pulmonary Infections

Pneumonia is one of the commonest serious respiratory infections caused by a wide variety of infectious agents, including bacteria, mycoplasma, fungi, parasites and viruses, resulting in infection, especially in the elderly and immunocompromised patient. Pneumonia is a significant cause of morbidity and mortality in patients of all ages. Bacterial pneumonia can be divided into two major categories: community-acquired pneumonia (CAP) and hospital-acquired pneumonia (HAP), including ventilator-acquired pneumonia (VAP) [47, 48]. Another severe respiratory disease affecting a significant proportion of the elderly population is chronic obstructive pulmonary disease(COPD), characterized by chronic obstruction of air flow through the airways or out of the lungs, with excess production of sputum.

The hypothesis of a correlation between pulmonary disease and oral diseases were advanced in 1968 for the first time, by Potter et al. that described the presence of dental diseases in subjects with pulmonary diseases [49]. Some studies have indicated that oral bacteria may enhance the risk of respiratory diseases, but bacteria alone are insufficient to cause disease. Other factors that inhibit the normal lung defence systems and increase susceptibility to respiratory infections must be taken into consideration [50].

Still, it has been shown that oral bacteria can enter in the lower respiratory tract, especially in the old and immune-compromised patient, by four possible ways:

- aspiration of oropharyngeal contents;
- inhalation in the lungs of infectious aerosols;
- spread of infection from contiguous sites;
- hematogenous spread from extrapulmonary sites of infection (gastrointestinal tract) [50, 51].

Dental plaque seem to be a rational source, or a reservoir of pulmonary pathogensparticularly in patients with periodontal disease and several mechanisms can to explain the propensity for pulmonary pathogens to colonize the oropharynx of susceptible patients:

1. periodontal pathogens such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* can be aspirated into the lung to initiate infections;
2. bacteria-derived enzymes found in saliva may modify the oral mucosa surfaces exposing receptors that favoured adhesion and colonization of pulmonary pathogens which are aspirated into the lung;

3. periodontitis-associated enzymes may degrade salivary pellicles, thus diminishing the protection of non-specific host defence against respiratory pathogens;
4. cytokines released from periodontal tissues may alter respiratory epithelium, promoting infection by respiratory pathogens[52].

Worthy of note is that some microorganisms unusual in the oral flora were isolated from dentures included respiratory pathogens such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *H. parainfluenzae*, *Escherichiacoli, Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobactercloacae* and *Pseudomonas aeruginosa*.The oral cavity of these subjects may serve as a reservoir of these pathogens for re-infection of the lung [16-20] and since, inhalation pneumonia is a common cause of death amongst the elderly debilitated, the role of the denture in harboring such potential pathogens may be significant [53].

Current evidence suggests that oral bacteria, poor oral hygiene and periodontitis may influence the initiation and course of pulmonary infections including community-, hospital-, and ventilator-acquired pneumonia[50]. Sufficient evidence is also available to support the relationship between poor oral hygiene and/or periodontitis and increased risk of pneumonia, particularly in hospitalized patients and institutionalized subjects. In these individuals, oral hygiene improvement obtained by mechanical and/or topical chemical disinfection (0.2% chlorhexidine) can decrease the incidence of pneumonia up to 40% [54].

According to such evidence oral hygiene of both dentate and edentoulus subjects will assume a pivotal role in prevention treatment of respiratory infection.

Periodontal Disease and Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an autoimmune inflammatory disease that affects several organs and systems. RA is associated with destruction of joint connective tissue and boneresulting in structural damage, disability and loss of articular function [55]. RA affecting 0.5–1% of the population [56, 57] and is associated with significant morbidity and an increased risk of premature death[58].

A number of clinical studies have indicated a potential positive association between periodontitis/tooth loss and RA [59-66], while one study observed no association between the occurrence of these diseases [67]. Still, these studies vary widely with respect to design, setting, and methods used to ascertain associations between rheumatic diseases and periodontitis. The majority of studies are relatively low-prevalence case control studies and the control subjects were often volunteers recruited from the staff at the study centers (for example, university or hospital staff) or were patients attending dental clinics. These patients may be unlikely representative of the general population (the source population for patients with rheumatic disease). This selection bias could have resulted in overestimation or underestimation of the association between RA and periodontitis/tooth loss. Another difficulty when evaluating the evidence for an association between rheumatic disease and periodontitis relates to a lack of consistent criteria used to define exactly and uniformly a case of periodontitis. However, in two studies a bidirectional relationship of RA and periodontitis have been proposed [61, 68].

Several infectious agents, including periodontal bacteria, have been implicated as causal to the etiology of RA [69-71]since CP may have systemic repercussions with increased inflammatory mediator levels and frequent transitory bacteremia forprotracted period of time. In this context, antibodies against oral anaerobic bacteria have been detected in serum[70, 72] and synovial tissue[69] of individuals with RA and in the same way, DNA from oral bacteria has been reported in the serum and synovial fluid of individuals with RA [71]. Bartold et al. [55] hypothesized the possibility of a common genetic trait predisposing to both conditions, in fact chronic periodontitis and RA present an imbalance between pro-inflammatory and anti-inflammatory cytokines, which is considered responsible for the tissue damage (both conditions are associated with destruction of bone mediated by inflammatory cytokines such as IL-1, TNF and PGE2). This association implies that certain features of the inflammatory response might be common to both diseases, in causal and non-causal pathway.

These pathways can be generally categorized into: those through which periodontitis is a causal factor in the pathogenesis of RA, resulting in increased incidence, activity or progression of RA;

1) those through which RA is a causal factor in the pathogenesis of periodontitis, resulting in increased incidence or progression of periodontitis;
2) non-causal pathways, in which environmental or host factors such as socioeconomic status, body mass index, alcohol consumption, poor oral hygieneand smoking, increase susceptibility to both RA and periodontitis[65].

Comprehension of a possible association between periodontitis and RA can be relevant for the medical care of patients affected by RA. Essentially, the association between periodontal diseaseand RA appears to be due to several common features:

1) both RA and PD are characterized by self-sustaining inflammation in a compartment adjacent to bone, in which inflammatory cells and other factors lead to common clinical symptoms (pain, swelling, tenderness) and, finally, to destruction of the adjacent bone;
2) RA and PD may share similar immunogenetics features [61].

In this context, immunogenetic studies on patients with RA have established an association with specific HLA antigens, in particular at the HLA DR4 locus[56]. The same genetic locus has been associated with development of severe periodontitis[73, 74]. Cells, enzymes and cytokines which determine the degree of tissue destruction all share a common pathologic process in RA and periodontitis. In both periodontal lesions and rheumatoid synovium, local immune responses are amplified with recruitment of inflammatory cells from the systemic circulation into the target tissue (gingival mucosa or synovialmembranae) [75].

Besides, RA may influence the pathogenesis of periodontitis through its motor and emotional damage [76]. In fact, motor impairment and disability in the upper extremities of the body reduced manual agility rendering more difficult the oral hygiene with consequent increase of the risk of caries, periodontitis and tooth loss [77]. The salivary flow reduction, due to medication or secondary Sjögren syndrome, may increase supragingival plaque formation in these individuals [78], such as psychological alterations found among RA patients have been suggested as risk indicators for periodontitis [79].

Epidemiologic, serologic, and animal model studies provided evidence that *Porphyromonas gingivalis*, the most important etiological agent of CP, might be involved in the onset and progression of RA [72, 80]. Although the etiology of RA remains unclear, the hypothesis suggests a new direction revealing that bacterial infection may play a role in loss of self-control and consequent development of an autoimmune disease. The linkage between *P. gingivalis* and RA comes to be realized in the studies of anti-cyclic citrullinated peptide (anti-CCP) antibody which is a highly specific marker for RA. This family of autoantibody includes antiperinuclear factor, antikeratin antibody, antivimentin, and antifilaggrin antibody and is generated by post-translational modification (citrullination) of protein-bound arginine by peptidylarginine deiminase (PAD) and casually, *P. gingivalis* is the only bacterium known to express a PAD enzyme and significantly associated with RA [80]. Deimination of arginine residues in autoantigenic proteins (profilaggrin/filaggrin, fibrinogen/fibrin, keratin, and vimentin) creates epitopes that are targeted by rheumatoid autoantibodies (anti-cyclic citrullinated peptide antibodies). Arginine is the most important of the amino acids associated with autoantigenicity of proteins. One report of a peptidylarginine deiminase expression in the bacterium *P. gingivalis*is been reported [81]. *Tannerella forsythensis* and *Treponema denticola* have also arginine-specific proteinase.

Rheumatoid factor (RF) has been detected in the gingiva, subgingival plaque, saliva, and serum of adult patients with PD [82, 83]. Oral pathogens promote production of RF, both directly (antibacterial) and indirectly (ligation of Toll-like receptor), both locally (in gingival tissue) and systemically (in serum) [84]. RFs have been identified as autoantibodies that react to the IgG molecule in the Fc region, and these antibodies may be of the IgM, A, G or E epitopes. *P. gingivalis* proteinase is responsible for the, epitope development in the RF Fc region. Bonagura et al. [85] identified the lysine and arginine amino acid sequences for the Fc region of the IgG molecule. Because *P. gingivalis* specifically decomposes lysine and arginine, the IgG3 CH2 and CH3 domains processed by *P. gingivalis* proteinase become powerful targets for the RF produced by rheumatoid cells [86].

Worthy of note is that, for several reasons, systemic bone loss is frequent in RA patients, resulting in an increased risk of osteoporosis[87].Inflammation in RA is associated with osteoclastogenesis, which results in generalized bone loss [88]. In addition, consequences of the pathologic process, including weight loss, reduced physical activity and use of medications such as steroids, all contribute to osteoporosis in RA. Several studies have reported associations between osteoporosis and periodontitis[89] and some studies indicate that maintenance of bone mineral density is associated with improved tooth retention in elderly men and women [90, 91]. In this context a paradox regard the medications used to manage the symptoms of RA and periodontitis/tooth loss exist, since most of the drugs used to treat RA would probably reduce the risk of developing periodontitis and/or its progression, rather than promote the onset or progression of periodontitis. Furthermore, there is robust evidence that NSAIDs are beneficial in the treatment of periodontitis [92]. A significant improvement in periodontal status was reported in patients with RA and periodontitis who received anti-TNF treatment [93].

Table 2. Characteristics and diabetes Type

Type	Characteristics
Type 1 diabetes mellitus (insulin-dependent diabetes or IDDM)	– It is the result of cellular-mediated immune β-cell destruction, in the islets of Langerhans of the pancreas, usually leading to absolute insulin deficiency. – Usually present in children and adolescents(5–10% of patients with diabetes). – Different etiologies: early exposure to cow's milk that may activate an autoimmune process, resulting in the destruction of the pancreatic β cells; abnormal response to an enterovirus infection. – In patients with type 1 diabetes the use of exogenous insulin it is essential for life; in fact in the absence of insulin these patients develop ketoacidosis, a life threatening condition. – If adequately not treated, these patients are likely to manifest the classic signs and symptoms of diabetes: polyuria (excessive urine output), polydipsia (excessive thirst) and polyphagia (excessive appetite), as well as pruritis, weakness and fatigue. – Systemic complications: Graves' disease, Hashimoto's thyroiditis, Addison's disease, vitiligo, celiac sprue, autoimmune hepatitis, myasthenia gravis, and pernicious anemia as part of the polyglandular autoimmune syndrome.
Type 2 diabetes mellitus (noninsulin-dependent diabetes or NIDDM)	– The onset is generally more gradual than at type 1, and it is often associated with obesity. – Type 2 diabetic patients have insulin resistance, which alters the utilization of endogenously produced insulin. – In many patients, especially early in the disease, insulin production is increased, resulting in hyper-insulinemia. With the progress of the condition, insulin production often decreases and patients have a relative insulin deficiency in association with peripheral insulin resistance. – Autoimmune destruction of β cells does not occur, and patients maintain the capacity for some insulin production. – Strong genetic component and is more prevalent among people with hypertension or dyslipidemia – High incidence (90-95% of the diabetic population).
Gestational diabetes mellitus (GDM)	– Glucose intolerance during pregnancy. – Complicated 90% of all pregnancy. – Women at high risk are those older than 25 years of age, with positive family history of diabetes, previous personal history of gestational diabetes mellitus, marked obesity, and members of high-risk ethnic groups like African- Americans, Hispanics, and American Indians. For at least 6 weeks after birth, the woman should receive an oral glucose tolerance test and be reclassified. Most women with GDM return to a norm-glycaemic condition after birth; – The history of GDM markedly increases the risk for subsequently developing type 2 diabetes such as the children of mothers with GDM are at greater risk of have obesity and diabetes as young adults. – The diagnosis of GDM can represent an unidentified pre-existing diabetic condition or a direct metabolic consequence of the hormonal changes.
Other specific types of diabetes	– -Included specific genetically defined forms of diabetes and diabetes associated with long-term or high-dose of drug use (steroid therapy for autoimmune diseases or post-organ transplantation), or other diseases such as: diseases of the exocrine pancreas (i.e. pancreatitis, trauma, infection, pancreatectomy, pancreatic carcinoma, cystic fibrosis and hemochromatosis), endocrinopathies (i.e. acromegaly, Cushing's syndrome, glucagonoma, and pheochromocytoma), viral infections (i.e. coxsackievirus B, cytomegalovirus, adenovirus, and mumps) and other genetic syndromes (i.e. Down's syndrome, Klinefelter's syndrome, Turner's syndrome, and Wolfram syndrome), that may cause β-cell destruction.

In conclusion, the majority of studies indicate that patients with RA have an increased prevalence of periodontitis and tooth loss. However, the strength of the association remains uncertain. Furthermore, all the available studies are cross-sectional and the temporality of the association between periodontitis and RA cannot be ascertained. The evidence-based medicine indicates that periodontitis might have a direct causal role by initiating and sustaining the immune-mediated inflammatory response in RA. Additional non-causal factors include genetic, environmental and behavioral factors are common to both conditions. Further pharmacological, physiological and behavioral factors are possible cause that might affect the incidence of periodontitis and/or its progression in individuals with RA

Periodontal Disease as a Risk Factorfor Diabetes

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia due to defective secretion or activity of insulin[94, 95]. In the current classification, the terms "insulin-dependent diabetes mellitus" and "noninsulin-dependent diabetes mellitus" are not used, because they relate to treatment rather than to the diagnosis. These criteria were modified to included the diagnosis of impaired fasting glucose (type 1 diabetes) and impaired glucose tolerance (type 2 diabetes). A conclusive diagnosis of diabetes mellitus is made by assessing glycated hemoglobin levels: in diabetes people, sequential fasting plasma glucose levels will be 7 mmol/L or more.

Diabetes mellitus can be classified into 1 of 4 large categories according to signs and symptoms[96](Table 2).

Generally, the complications of diabetes are related to long-term elevation of blood glucose concentrations (hyperglycemia), that results in the formation of advanced glycation end-products (AGEs). This products induced marked changes in cells and extracellular matrix components including abnormal endothelial cell function, capillary growth and vessel proliferation. The accumulation of AGEs increases the intensity of the immune-inflammatory response to various pathogens, because inflammatory cells such as monocytes and macrophages have receptors for AGEs. Interactions between AGEs and their receptors on inflammatory cells result in the increased production of proinflammatory cytokines such as IL-1β and TNF-ά. Accumulation of AGEs in the plasma and tissues of diabetic patients has been linked, to peripheral neuropathy, retinal degeneration, renal insufficiency, atherosclerosis, and microangiopathy [97, 98].

During the past 50 years, more than 200 articles have reported a higher incidence and severity of periodontal disease in diabetic patients, while in the last decade other studies has shown a two-way relationship between chronic periodontitis and diabetes mellitus and definite periodontitis as the sixth complication of diabetes [99]. Many of the mechanisms by which diabetes influences the periodontium are similar to the pathophysiology of the classic microvascular and macrovascular diabetic complications.

Diabetes may result in a damage of neutrophil adherence, chemotaxis, and phagocytosis, which may facilitate bacterial persistence in the periodontal pocket and significantly increase periodontal destruction. While neutrophils are often hypo-functional in diabetes, these patients may have a hyper-responsive monocyte/macrophage phenotype, resulting in significantly increased production of pro-inflammatory cytokines and mediators. This hyper-

inflammatory response results in elevated levels of pro-inflammatory cytokines in the gingival crevice fluid. Elevated levels of inflammatory mediators, including PGE_2, IL-1β, and TNF-ά in gingival exudates, are associated with increased severity of periodontal disease in patients with diabetes and AGEs-enriched gingival tissue has greater vascular permeability, greater breakdown of collagen fibers and shows accelerated destruction of both non-mineralized connective tissue and bone[100]. Moreover, the sites affect by periodontitis in diabetic patients contain the same bacterial species as infected sites in patients without diabetes. Similar constitution of the subgingival flora might indicate that the cause for increased prevalence and severity of periodontitis in diabetics lies in the host response. Increased levels of glucose in sulcular fluid may adversely influence healing processes and local response to microorganisms. In this context, in people with diabetes who have poor glycaemic control (high blood sugar levels), the risk of developing periodontal infection is much greater (from 2 to 4 times) than non-diabetic people. Diabetic patients who have good control over blood sugar levels (good glycaemic/metabolic control) can prevent or delay the onset and slow the progression of the complications associated with diabetes, particularly retinopathy, nephropathy, and neuropathy. The same is true for delaying the onset or slowing the progression of periodontitis [99].

In diabetic patient, glycaemic control is complicated from the constant reservoir of gram-negative anaerobic bacteria that sit at the bottom of the gum pockets producing infection and low grade inflammation in total body. An important aspect of the diabetes-periodontal disease relationship is that chronic periodontal infection causes systemic inflammation with consequent enhances insulin resistance and hyperglycemia. Since, people with diabetes, especially those with poor glycemic control, accumulate high levels of AGEs in the tissues, including the periodontium this interaction may be the cause of the marked elevation in gingival crevicular fluid levels of IL-1β, and TNF- seen in subjects with diabetes respect to without diabetes people, and it may contribute to the increased prevalence and severity of periodontal disease in diabetics [101].

Evidence suggests that chronic periodontitis can induce or perpetuate an elevated systemic chronic inflammatory state, as reflected in increased serum C-reactive protein, IL-6, and fibrinogen levels seen in many people with periodontitis [102, 103]. Investigations also found that the status of periodontal health in diabetic patients with good or moderate control of their glycaemic condition was similar to that in the non diabetic controls; those with poor control had more attachment loss and were more likely to exhibit recurrent disease [104-107]. Furthermore, it has been shown that periodontal treatment directed at elimination of periodontal pathogens and at reduction of inflammation may have a positive impact on glycaemic control by re-establishing insulin sensitivity in poorly controlled patients with diabetes and that effective control of periodontal infection in patients with diabetes may reduce the level of AGEs in the serum [104, 106, 108]. From this, it is possible conclude that preventionand control of periodontal disease must be considered an integralpart of diabetes control; so the effective control of periodontal infection in patients with diabetes may reduce the level of AGEs in the serum [109].

Periodontal Disease and Pancreatic Cancer

Cancer of the pancreas, the fourth leading cause of cancer death in the United States, is a rapidly fatal tumor[110]. Smoking is the only well documented modifiable risk factors for pancreatic cancer[111], although data suggest that diabetes, obesity and insulin resistance are also associated with risk [112]. In addition, alcohol consumption is not an established risk factor for pancreatic cancer, but there is a very strong association between alcohol consumption and chronic pancreatitis, and the latter has been associated with an high risks for pancreatic cancer [113]. These associations suggested that chronic inflammation of the pancreas is associated with a greater risk of pancreatic cancer although the inflammatory mediators that lead to the development of this remain poorly defined [114]. Inflammation may increase cellular proliferation and mutagenesis, reduce adaptation to oxidative stress, promote angiogenesis, inhibit apoptosis, and increase secretion of inflammatory mediators[115].

Since periodontitis is a chronic bacterial infection which results in inflammation of the gingivae, leading to the gradual destruction of periodontal tissues and alveolar bone supporting the teeth, a few authors have suggested a possible positive association between periodontitis and cancer risk in different tissues [116-122]. In particular, chronic periodontitis has been associated with increased risk for gastrointestinal diseases, including oral [123], intestinal [124], esophageal [124], gastric [119] and pancreatic tumors[116].

Periodontitis may promote pancreatic carcinogenesis, by means systemic inflammation[125, 126], or alternatively, through increased production of carcinogens, namely nitrosamines[117, 119]. Nitrosamines and gastric acidity have been hypothesized to have an important role in pancreatic cancer[127] and tooth loss that occurs through poor dental hygiene may be a marker for more deleterious gastrointestinal flora and, consequently, greater endogenous nitrosation. Endogenous formation of nitrosamines in the oral cavity in persons with poor oral hygiene is 8-fold that in persons with good hygiene [128]. In fact, individuals with periodontal disease have elevated levels of oral bacteria and have very higher nitrosamine levels in oral cavity [128] due to nitrate-reducing bacteria, including *H. pylori*[129]. The association between *H. pylori* infection and pancreatic cancer has been investigated inthree studies [130-132], but this association could not be confirmed.

Periodontal Disease and Metabolic Syndrome

Metabolic syndrome (MetS) is defined as the contemporary presence of hypertension, atherogenic lipid profiles altered (hyper-triglyceridemia and low high-density lipoprotein (HDL)-cholesterol), obesity (particularly central adiposity) and insulin resistance. A proinflammatory and procoagulant state may also coexist in this syndrome, with elevation of C-reactive protein and fibrinogen[133, 134]. Subjects with the MetS have an increased risk for type 2 diabetes[135] and cardiovascular diseases [136]

There is evidence suggesting that this chronic inflammatory state is associated with endothelial dysfunction, which might contribute to the increased cardiovascular risk of people affected by this disorder and with the increased risk of type 2 diabetes[137]**.** Patients with MetS had poor periodontal health and chronic periodontitis was associated with MetS,

independent of other risk factor [22, 138]. As both periodontitis and MetS are associated with systemic inflammation and insulin resistance, these two diseases may be linked through a common pathophysiological pathway (increased serum levels of products derived from oxidative damage) and a bidirectional association it has been hypothesized [139-141].

Periodontal Disease andAlzheimer Disease

Alzheimer's disease (AD) is a progressive neurodegenerative disorder affecting aged people and characterized by irreversible cognitive and physical deterioration. The etiology of AD is multifactorial with possible inclusion of well-known vascular risk factors and infectious agents [134, 142]. Inflammation has been suggested have to a role in the pathophysiology of AD [143], and certain pathogens may play a role in the acceleration of the inflammatory response and increased oxidation, causing inflammatory reactions and oxidative damage with consequent vascular changes [144, 145].

In view of the bacterial profile associated with periodontitis it is not surprising to hypothesize that periodontitis may have significant systemic effects in the brain. A number of studies have reported on the oral health status of AD patients; [146-153][154, 155] and have shown that patients with dementia are more likely to have poor oral health. Other authors [156-158] have alsoattempted to relate oral disease to the successive risk of developing cognitive deficit. The progression of dementia is accompanied by a gradual inability to perform self-care, including adequate oral hygiene, due to self-neglect and loss of cognitive and motor skills[159], and so patients with AD have more gingival plaque, bleeding, and calculus compared to age- and gender-matched adults healthy [152], and submandibular saliva production is damaged in medicated persons with AD [160]. Insufficient and inadequate oral hygiene, impaired access to professional oral examination and treatment, and frequent medical management with psychotropic medications that cause salivary dysfunction, all combine to negatively affect oral health and function.

Recently, Kamer et al [25] have proposed two mechanisms that may be involved in the periodontal disease-induced progression of AD.

In the first mechanism, bacteria responsible of moderate or severe periodontitis, such as spirochetesand particularly *Treponema denticola,* may spread to the brain for via neuronal pathways[161]. Riviere and colleagues [162] suggested that this bacteria may utilize branches of the trigeminal nerve to reach the brain. In fact, in their postmortem examination of brain tissues, they identified antigens of oral treponemes more often in samples from subjects with AD (14 of 16) than in samples from control subjects (4 of 18). Once in the brain, periodontal bacteria that are rich in LPS or their products are capable of stimulating cytokine production. These cytokines may act on the already primed glial cells resulting in an amplified reaction and possible progression of AD [163].

The second mechanism implies that periodontal disease-derived inflammatory molecules increase brain inflammation; in fact periodontal pathogens into the systemic blood stream may result in a sustained and continuous elevation of inflammatory products within the circulation i.e. inflammatory cytokines, including IL-1β, IL-6, IL8, TNF-α, and C-reactive protein. This inflammation within the central nervous system is thought to play a pivotal role in AD and has been hypothesized that peripheral infection/inflammation might alter the

inflammatory state in the brain [164]. The potential role of cytokines in neurological changes relating to psychiatric disorders, including AD, has been highlighted in the literature [165], and IL-6, has been demonstrated in and around senile plaques [143]. Several studies have linked polymorphisms (variations) in the IL-1 gene family to periodontitis[166] and AD [167, 168]. Although these polymorphisms are found at different loci, it possible consider that these polymorphisms reflect a hyper-inflammatory genotype that could be an trait common to people with periodontits and people with dementia[169, 170]. If periodontal infection would contribute to the course of AD, this could have important implications for future prevention, and perhaps treatment, of AD [134].

Periodontal Disease and Chronic Kidney Disease

Chronic kidney disease (CKD) is defined as a progressive decline in renal function associated with a reduced glomerular filtration rate (as measured clinically by the creatinine clearance) [171]. Traditional risk factors for CKD include age > 60 years, diabetes, poor glycaemic control, obesity, macroalbuminuria, smoking, high serum level of CRP, elevated total cholesterol, low levels of high-density lipoprotein (HDL) cholesterol, race/ethnicity, gender and income/poverty; non-traditional risk factors that may contribute to CKD include periodontal disease and instruction level [171, 172].

The effects of CKD on oral tissues, especially in children, including xerostomia, delayed tooth eruption, calcifications leading to obliteration of pulp chamber and canals, enamel hypoplasia, decreased caries rates and altered salivary pH levels are well-documented [173]. Besides in patients with CKD receiving haemodialysis it is characteristic the malodor or ‘bad breath’ and a metallic taste which are due to the high urea content in saliva and its subsequent breakdownto ammonia[174]. Dental management in CKD patients is complicated by some systemic consequences (e.g. anaemia, crisis of bleeding and cardiovascular or endocrine diseases). Moreover, untreated dental infection in immune-suppressed individuals can potentially contribute to morbidity and transplant rejection[175]. For this reason, many transplant centres plan a dental check in their pre-transplant protocol.

Specific effects of CKD and renal replacement therapy on periodontal tissues include gingival overgrowth and increased gingival inflammation[176]. Recent studies suggest that chronic periodontitis may contribute to the overall chronic systemic inflammatory burden and therefore may have consequences in the management of end-stage renal disease in patients on haemodialysis or maintenance therapy[23, 177]. Interventional studies are needed to assess the effect of periodontal treatment on systemic inflammation and CKD patients in this population.

Periodontal Disease and Osteoporosis

Osteoporosis (OP) is a systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in fragility and susceptibility to fracture of bones[178]. In the past, OP was considered a physiological

process associated with ageing, but today it is viewed as a heterogeneous chronic systemic condition which can occur in any age and its aetiology is attributed to various endocrine, metabolic and individual factors.Furthermore, recently OP has received increasing attention in relation to the susceptibility to PD in postmenopausal women[179, 180]. The production of estrogens changes drastically at menopause leading to OP in skeletal bones characterized by the loss of bone mass and reduction of bone density with a consequent increase in bone fragility and susceptibility to fracture. Total skeletal mass reduction in postmenopausal women may include jawbones, particularly the mandible[181]. A number of studies showed that bone changes in OP are associated with loss of periodontal attachment, loss of teeth, and height of residual ridge [182, 183]and an hypothesis of a bi-directional aetiological mechanism has been advanced.In the presence of severe generalized OP, reduced bone mineral density implies an increased porosity of the alveolar bone at the jaw bone, an altered trabecular pattern and a more rapid bone resorption in presence of chronic periodontitis Furthermore, bacteria, directly through the release of toxins, or indirectly through the release of inflammatory mediators, alter the normal homeostasis of bone tissue, increasing osteoclastic activity and reducing bone density [19]. The loss of bone mass that is observed in these two conditions appears to be modulated by systemic and local factors, sometimes common, as are many risk factors (Table 3). However, a definitive evidence is lacking.

Table 3. The risk factors for osteoporosis and periodontal disease

Risk Factors For Osteoporosis	**Common Risk Factors**	**Risk Factors For Periodontal Disease**
Systemic Risk Factors - Female gender - Low body weight/stature/very thin body - Premature menopause (<45 years) - Extended periods of amenorrhea - Lack of estrogen/testosterone - Previous osteoporotic fracture of the hip, spine or wrist - Family history of osteoporotic fracture	Systemic Risk Factors - Ageing - Gender - Race - Ethnicity - Genetic predisposition	Syndromes and Hereditary Diseases With Alteration of Connective Tissue - Down syndrome - Ehlers-Danlos syndrome - Papillon-Lefèvre syndrome
Malnutrition and Malabsorbiment Conditions Deficient intake of calcium, phosphorus, sodium magnesium, vitamins D, K, B6, B12 High consumption of animal protein, coffee, soda, spinach, wheat derivatives Anorexia Cystic fibrosis Inflammatory bowel disease (Chron's disease and ulcerative colitis) - Gastrectomy/gastro intestinal bypass - Celiac disease	Behavioral and Environmental Risk Factors - Smoking - Abuse of alcohol (more than 2/3 units of alcohol per day) - Low socio-economic status	Syndromes and Hereditary Diseases With Phagocytic Disfunction - Neutropenia (Congenital neutropenia, Chronic benign neutropenia, Cyclic neutropenia) - Felty Syndrome - Functional disorders of adhesion - Leukocyte adhesion deficiency - Functional disorders of chemotaxis - Hypergammaglobulinemia syndrome (Job's syndrome) - Chediak-Higashi syndrome - Lazy leukocyte syndrome - Disorders of phagocytosis - Deficiency of myeloperoxidase - Chronic granulomatous disease - Deficit of specific granules
Drugs Aluminum-containing antacids Antiseizure medications Aromatase inhibitors Cancer chemotherapeutic drugs Cyclosporine A and Tracolimus Systemic glucocorticoid therapy for > 3 months with ≥ 5mg/die cortisone and	Acquired Immunodeficiency (AIDS/HIV infection)	Drugs - Calcium antagonists, - Oral contraceptives - Protease inhibitors

prednisone Gonadotropin releasing hormone (GnRH) Lithium Medroxyprogesterone acetate for contraception Methotrexate Proton pump inhibitors Selective serotonin reuptake inhibitors Tamoxifen (premenopausal use) Thiazolidenediones Thyroid hormones in excess Loop diuretics -Anticonvulsivant heparin long term therapy		
Hereditary Skeletal Diseases Osteogenesis imperfecta Hypophosphatasia	Endocrine and Metabolic Diseases - Type 1 diabetes mellitus (IDDM)	Stress
Endocrine and Metabolic Diseases Hypogonadism, Hyperparathyroidism Hyperthyroidism, Cushing Syndrome Acidosis - Gaucher's Disease	Drugs - Antiepileptics - Cyclosporine	Hormone Variations - Puberty, menstruation, pregnancy, menopause
Bone Marrow Diseases - Multiple myeloma Lymphoma / leukemia Mastocytosis - Thalassemia		Osteoporosis and Osteopenia
Other Medical Conditions Chronic renal insufficiency Hypercalciuria Systemic Lupus Erythematosus Breast cancer Emphysema Female athlete triad Idiopathic scoliosis Kidney disease Multiple sclerosis Organ transplants Parkinson's disease Post-polio syndrome Prostate cancer Rheumatoid arthritis Severe liver disease (including biliary cirrhosis) Thyrotoxicosis Depression Prolungate immobility (trauma of spinal cord, stroke, muscular dystrophy, ankylosing spondylitis)		Radiotherapy
Other Factors - Inactive lifestyle/Physical inactivity		Behavioral and Environmental Risk Factors - Subgingival bacterial plaque - Poor oral hygiene
		Microbiological Risk Factors - Aggregatibacter actinomycetemcomitans - Tannerella forsythis - Porphyromonas gingivalis - Virus
		Local Risk Factors - Tooth-related risk factor (mucogingival anomalies, presence of iatrogenic factors) - Occlusal trauma

About this association it should be noted that:

1) OP is characterized by loss of bone tissue in all part of the body including the jaw bones;
2) in OP patient, trabecular part of skeletal segments present a reduced density resulting in increased porosity and rapid alveolar bone resorption by means of periodontal pathogens;
3) periodontal pathogens through their toxins elicited the mechanisms of release of pro-inflammatory cytokines, alter the systemic homeostasis of bone tissue, promoting osteoclastic activity and reducing bone density [19].
4) dentist may be of primary importance in the early diagnosis of OP, with the opportunity to assess the health of the entire skeleton of the patient through the panoramic radiograph.

Periodontal Disease and Cardiovascular Disease

Over the last 15 years the role of CP has been largely investigated as a risk factor for cardiovascular disease (CVD) [184]. In the past, cardiovascular risk factors were reported in the Framingham Heart Study [185]; later, Matthews [186] suggested that the classic risk factors (hypertension, smoking, diabetes mellitus) can only account for ⅔ of the incidence of CVDs cases, and that unrecognized risk factors should be involved in the pathogenesis of CVDs. Several mechanisms have been proposed in the attempt to establish a relationship between these apparently independent diseases: they include the direct participation of oral bacteria in the pathogenesis of atherosclerotic plaques or the possible involvement of systemic inflammatory mediators derived from oral and, in particular, periodontal infection in the development of atherosclerotic complications[184, 187, 188].

Under this point of view, there are sufficient data to consider the species*Aggregaticbacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsithya* as key pathogens for CP and it is well documented that periodontal pathogens and their components (toxins) can penetrate the epithelial barrier of the periodontal tissues and get systemic spreading through the blood stream. By this dynamic, periodontal pathogens infect directly the vascular endothelium, and they have been found to be able to infect atherosclerotic plaques, causing inflammation and plaque instability up to a myocardial ischemia[189-191]. Indirectly, periodontal pathogens elicit a great production of pro-inflammatory mediators such as interleukinswhich could induce secretion in the liver of acute phase reactants, such as C-reactive protein and fibrinogen which can contribute to the formation of atheroma and increase the risk for thrombogenesis [192].

A recent consensus concluded that chronic periodontitis may be an independent risk factor for future cardiovascular events [184], although the relation is relatively weak, conferring a 24% to 35% increased risk (OR: 1.24 to 1.35) [187]. Furthermore, it should be noted that the treatment of chronic periodontitis decreases systemic markers of inflammation and improves endothelial dysfunction in systemically healthy subjects [193]. However, there is no definitive evidence that cardiovascular disease events can be prevented with periodontal therapy.

Conclusion

The interactions between periodontal infections and systemic health represent a new and crucial area for research. The current evidence suggests that inflammation due to periodontal infections may not be limited to the immediate oral environment but can have systemic effects. More well-controlled intervention studies are warranted to confirm that periodontal infections could be true risk factors for important systemic diseases and that the management of relevant medical conditions could be improved by periodontal treatment and regular maintenance care. However, on the basis of available knowledge, medical community should be aware of the potential negative effects of periodontal infections on systemic health.

References

[1] Miller W: The human mouth as a focus of infection. *Dental Cosmos* 1891, 33:689-713.

[2] Billings F: Chronic focal infections and their etiologic relations to arthritis and nephritits. *Archives of internal medicine* 1912, 9:484- 498.

[3] Billings F: Focal infection: Its broader application in the etiology of disease. *JAMA* 1914;:899-903.

[4] Rosenow EC: Results of experimental studies on focal infection and elective localization. *The Medical clinics of North America*1922, 5:573-592.

[5] Rosenow EC: Transmutations within the streptococcus-pneumococcus group. *The Journal of infectious diseases* 1914, 14:1-32.

[6] Hughes R: Focal infection revisited. *Br J Rheumatol* 1994, 33:370-377.

[7] Gibbons R: Germs, Dr. Billings, and the theory of focal infection. *Clin Infect Dis* 1998, 27:627-633.

[8] Debelian GJ, Olsen I, L. T: Systemic diseases caused by oral microorganisms. *Endod Dent Traumatol* 1994, 10:57-65.

[9] Simon Camilleri, Audrey Camilleri, George Camilleri, Josette Camilleri, Joseph Camilleri, Alex Cassar, Mulligan K: Dentistry. Clinical update. *Malta Medical Journal* 2004, 16 (October)(03).

[10] Cecil RL, Angevine DM: Clinical and experimental observations on focal infection, with an analysis of 200 cases of rheumatoid arthritis. *Ann Intern Med* 1938, 12:577-584.

[11] American Association of Endodontists. Oral disease and systemic health: What is the connection? Endodontics: Colleagues For Excellence. Spring/Summer 2000.

[12] British Dental Association. Endodontic treatment and general health. BDA Fact Files 1996; April.

[13] Offenbacher S: Periodontal diseases: pathogenesis. *Annals of Periodontology* 1996, 1(1):821-878.

[14] Bahrani-Mougeot FK, Thornhill M, Sasser H, Marriott I, Brennan MT, Papagerakis S, Coleman S, Fox PC, Lockhart PB: Systemic host immuno-inflammatory response to dental extractions and periodontitis. *Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics* 2008, 106(4):534-541.

[15] Carmona IT, Diz Dios P, Scully C: An update on the controversies in bacterial endocarditis of oral origin. *Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics* 2002, 93(6):660-670.

[16] Guntheroth WG: How important are dental procedures as a cause of infective endocarditis? *The American journal of cardiology* 1984, 54(7):797-801.

[17] Li X, Kolltveit KM, Tronstad L, Olsen I: Systemic diseases caused by oral infection. *Clin Microbiol Rev* 2000, 13(4):547-558.

[18] Page RC: The pathobiology of periodontal diseases may affect systemic diseases: inversion of a paradigm. *Annals of periodontology / the American Academy of Periodontology* 1998, 3(1):108-120.

[19] Kuo LC, Polson AM, Kang T: Associations between periodontal diseases and systemic diseases: a review of the inter-relationships and interactions with diabetes, respiratory diseases, cardiovascular diseases and osteoporosis. *Public Health* 2008, 122(4):417-433.

[20] Offenbacher S., Katz V., Fertik G., Collins J., Boyd D., Maynor G., McKaig R., Beck J.: Periodontal disease as a possible risk factor for preterm low birth weight. *J Periodontol* 1996, 67:1103-1113.

[21] Michaud DS, Joshipura K, Giovannucci E, Fuchs CS: A Prospective Study of Periodontal Disease and Pancreatic Cancer in US Male Health Professionals. *J Natl Cancer Inst 2007* 2007, 99:171 - 175.

[22] Li P, He L, Sha YQ, Luan QX: Relationship of metabolic syndrome to chronic periodontitis. *Journal of periodontology* 2009, 80(4):541-549.

[23] Craig RG: Interactions between chronic renal disease and periodontal disease. *Oral diseases* 2008, 14(1):1-7.

[24] Bartold PM, Marshall RI, Haynes DR: Periodontitis and rheumatoid arthritis: a review. *J Periodontol* 2005, 76(supplement 11):2066-2074.

[25] Kamer AR, Dasanayake AP, Craig RG, Glodzik-Sobanska L, Bry M, de Leon MJ: Alzheimer's disease and peripheral infections: the possible contribution from periodontal infections, model and hypothesis. *J Alzheimers Dis* 2008, 13(4):437-449.

[26] Paster BJ, Olsen I, Aas JA, FE D: The breadth of bacterial diversity in the human periodontal pocket and other oral sites. *Periodontol 2000* 2006, 42:80-87.

[27] Haffajee AD, Socransky SS: Microbial etiological agents of destructive periodontal diseases. *Periodontol 2000* 1994, 5:78-111.

[28] Hentges DJ: The anaerobic microflora of the human body. *Clin Infect Dis* 1993, 16 Suppl 4:S175-180.

[29] Haraszthy VI, Zambon JJ, Trevisan M, Zeid M, RJ. G: Identification of periodontal pathogens in atheromatous plaques. *J Periodontol 2000* 71(Oct; 10):1554-1560.

[30] Garcia RI, Henshaw MM, Krall EA: Relationship between periodontal disease and systemic health. *Periodontol 2000* 2001, 25:21-36.

[31] Seymour GJ, Ford PJ, Cullinan MP, Leishman S, Yamazaki K: Relationship between periodontal infections and systemic disease. *Clin Microbiol Infect* 2007, 13 Suppl 4:3-10.

[32] Robert L Goldenberg, Jennifer F Culhane, Jay D Iams, Romero R: Preterm Birth 1. Epidemiology and causes of preterm birth. *Lancet* 2008, 371:75-84.

[33] Romero R, Mazor M, Wu YK, Sirtori M, Oyarzun E, Mitchell MD, Hobbins JC: Infection in the pathogenesis of preterm labor. *Seminars in perinatology* 1988, 12(4):262-279.

[34] McDonald HM, O'Loughlin JA, Jolly P, Vigneswaren R, PJ. M: Vaginal infection and preterm labor. *Br J Obstet Gynecol Surv* 1991, 98::427- 435.

[35] Pizzo G, La Cara M, Conti Nibali M, Guiglia R: Periodontitis and preterm delivery. A review of the literature. *Minerva stomatologica* 2005, 54(1-2):1-14.

[36] Gibbs RS, Romero R, Hillier SL, Eschenbach DA, Sweet RL: A review of premature birth and subclinical infection. *Am J Obstet Gynecol* 1992, 166(5):1515-1528.

[37] Bosnjak A, Relja T, Vucicevic-Boras V, Plasaj H, Plancak D: Pre-term delivery and periodontal disease: a case-control study from Croatia. *Journal of clinical periodontology* 2006, 33(10):710-716.

[38] Offenbacher S, Jared HL, O'Reilly PG, Wells SR, Salvi GE, Lawrence HP ea: Elevated human IgM suggests in utero exposure to periodontal pathogens. . *Journal of dental research* 1999, 78::2191.

[39] X, Buekens P, Vastardis S, Yu SM: Periodontal disease and pregnancy outcomes: state-of-the-science. *Obstetrical & gynecological survey*2007, 62(9):605-615.

[40] Boggess KA, Lieff S, Murtha AP, Moss K, Beck J, Offenbacher S: Maternal periodontal disease is associated with an increased risk for preeclampsia. *Obstet Gynecol* 2003, 101(2):227-231.

[41] Vettore MV, Leal M, Leao AT, da Silva AM, Lamarca GA, Sheiham A: The relationship between periodontitis and preterm low birthweight. *Journal of dental research* 2008, 87(1):73-78.

[42] Xiong X, Buekens P, Fraser WD, Beck J, Offenbacher S: Periodontal disease and adverse pregnancy outcomes: a systematic review. *Bjog* 2006, 113(2):135-143.

[43] Vergnes JN, Sixou M: Preterm low birth weight and maternal periodontal status: a meta-analysis. *Am J Obstet Gynecol* 2007, 196(2):135 e131-137.

[44] Srinivas SK, Sammel MD, Stamilio DM, Clothier B, Jeffcoat MK, Parry S, Macones GA, Elovitz MA, Metlay J: Periodontal disease and adverse pregnancy outcomes: is there an association? *Am J Obstet Gynecol* 2009, 200(5):497 e491-498.

[45] Macones GA, Parry S, Nelson DB, Strauss JF, Ludmir J, Cohen AW, Stamilio DM, Appleby D, Clothier B, Sammel MD *et al*: Treatment of localized periodontal disease in pregnancy does not reduce the occurrence of preterm birth: results from the Periodontal Infections and Prematurity Study (PIPS). *Am J Obstet Gynecol* 2010, 202(2):147 e141-148.

[46] Newnham JP, Newnham IA, Ball CM, Wright M, Pennell CE, Swain J, Doherty DA: Treatment of periodontal disease during pregnancy: a randomized controlled trial. *Obstetrics and gynecology* 2009, 114(6):1239-1248.

[47] Toews GB: Nosocomial pneumonia. *The American journal of the medical sciences* 1986, 291(5):355-367.

[48] Brown J: Oral biofilms, periodontitis and pulmonary infections. *Oral diseases* 2007, 13:513-514.

[49] Potter RT, Rotman F, Fernandez F, McNeill TM, Chamberlain JM: The bacteriology of the lower respiratory tract. Bronchoscopic study of 100 clinical cases. *The American review of respiratory disease* 1968, 97(6):1051-1061.

[50] Paju S, Scannapieco FA: Oral biofilms, periodontitis, and pulmonary infections. *Oral diseases* 2007, 13(6):508-512.

[51] Fiddian-Green RG, Baker S: Nosocomial pneumonia in the critically ill: product of aspiration or translocation? *Critical care medicine* 1991, 19(6):763-769.

[52] Scannapieco FA, Wang B, Shiau HJ: Oral bacteria and respiratory infection: effects on respiratory pathogen adhesion and epithelial cell proinflammatory cytokine production. *Annals of periodontology / the American Academy of Periodontology* 2001, 6(1):78-86.

[53] Wang Z, Zhou X, Zhang J, Zhang L, Song Y, Hu FB, Wang C: Periodontal health, oral health behaviours, and chronic obstructive pulmonary disease. *Journal of clinical periodontology* 2009, 36(9):750-755.

[54] Tantipong H, Morkchareonpong C, Jaiyindee S, Thamlikitkul V: Randomized controlled trial and meta-analysis of oral decontamination with 2% chlorhexidine solution for the prevention of ventilator-associated pneumonia. *Infect Control Hosp Epidemiol* 2008, 29(2):131-136.

[55] Bartold PM, Marshall RI, Haynes DR: Periodontitis and rheumatoid arthritis: a review. *Journal of periodontology* 2005, 76(11 Suppl):2066-2074.

[56] Gabriel SE: The epidemiology of rheumatoid arthritis. *Rheumatic diseases clinics of North America*2001, 27(2):269-281.

[57] Silman AJ, Pearson JE: Epidemiology and genetics of rheumatoid arthritis. *Arthritis research* 2002, 4 Suppl 3:S265-272.

[58] Gabriel SE, Crowson CS, O'Fallon WM: Mortality in rheumatoid arthritis: have we made an impact in 4 decades? *The Journal of rheumatology* 1999, 26(12):2529-2533.

[59] Albandar JM: Some predictors of radiographic alveolar bone height reduction over 6 years. *Journal of periodontal research* 1990, 25(3):186-192.

[60] Kasser UR, Gleissner C, Dehne F, Michel A, Willershausen-Zonnchen B, Bolten WW: Risk for periodontal disease in patients with longstanding rheumatoid arthritis. *Arthritis and rheumatism* 1997, 40(12):2248-2251.

[61] Mercado F, Marshall RI, Klestov AC, Bartold PM: Is there a relationship between rheumatoid arthritis and periodontal disease? *Journal of clinical periodontology* 2000, 27(4):267-272.

[62] Mercado FB, Marshall RI, Klestov AC, Bartold PM: Relationship between rheumatoid arthritis and periodontitis. *Journal of periodontology* 2001, 72(6):779-787.

[63] Abou-Raya A, Abou-Raya S, Abu-Elkheir H: Periodontal disease and rheumatoid arthritis: is there a link? *Scandinavian journal of rheumatology* 2005, 34(5):408-410.

[64] Marotte H, Farge P, Gaudin P, Alexandre C, Mougin B, Miossec P: The association between periodontal disease and joint destruction in rheumatoid arthritis extends the link between the HLA-DR shared epitope and severity of bone destruction. *Annals of the rheumatic diseases*2006, 65(7):905-909.

[65] Pischon N, Pischon T, Kroger J, Gulmez E, Kleber BM, Bernimoulin JP, Landau H, Brinkmann PG, Schlattmann P, Zernicke J *et al*: Association among rheumatoid arthritis, oral hygiene, and periodontitis. *Journal of periodontology* 2008, 79(6):979-986.

[66] de Pablo P, Dietrich T, McAlindon TE: Association of periodontal disease and tooth loss with rheumatoid arthritis in the US population. *The Journal of rheumatology* 2008, 35(1):70-76.

[67] Sjostrom L, Laurell L, Hugoson A, Hakansson JP: Periodontal conditions in adults with rheumatoid arthritis. *Community dentistry and oral epidemiology*1989, 17(5):234-236.

[68] Ribeiro J, Leao A, Novaes AB: Periodontal infection as a possible severity factor for rheumatoid arthritis. *Journal of clinical periodontology* 2005, 32(4):412-416.

[69] Moen K, Brun JG, Madland TM, Tynning T, Jonsson R: Immunoglobulin G and A antibody responses to Bacteroides forsythus and Prevotella intermedia in sera and synovial fluids of arthritis patients. *Clinical and diagnostic laboratory immunology* 2003, 10(6):1043-1050.

[70] Ogrendik M, Kokino S, Ozdemir F, Bird PS, Hamlet S: Serum antibodies to oral anaerobic bacteria in patients with rheumatoid arthritis. *MedGenMed* 2005, 7(2):2.

[71] Moen K, Brun JG, Valen M, Skartveit L, Eribe EK, Olsen I, Jonsson R: Synovial inflammation in active rheumatoid arthritis and psoriatic arthritis facilitates trapping of a variety of oral bacterial DNAs. *Clinical and experimental rheumatology* 2006, 24(6):656-663.

[72] Mikuls TR, Payne JB, Reinhardt RA, Thiele GM, Maziarz E, Cannella AC, Holers VM, Kuhn KA, O'Dell JR: Antibody responses to Porphyromonas gingivalis (P. gingivalis) in subjects with rheumatoid arthritis and periodontitis. *International immunopharmacology* 2009, 9(1):38-42.

[73] Katz J, Goultschin J, Benoliel R, Brautbar C: Human leukocyte antigen (HLA) DR4. Positive association with rapidly progressing periodontitis. *Journal of periodontology* 1987, 58(9):607-610.

[74] Bonfil JJ, Dillier FL, Mercier P, Reviron D, Foti B, Sambuc R, Brodeur JM, Sedarat C: A "case control" study on the role of HLA DR4 in severe periodontitis and rapidly progressive periodontitis. Identification of types and subtypes using molecular biology (PCR.SSO). *Journal of clinical periodontology* 1999, 26(2):77-84.

[75] Weissmann G: Pathogenesis of rheumatoid arthritis. *J Clin Rheumatol* 2004, 10(3 Suppl):S26-31.

[76] Persson LO, Berglund K, Sahlberg D: Psychological factors in chronic rheumatic diseases--a review. The case of rheumatoid arthritis, current research and some problems. *Scandinavian journal of rheumatology* 1999, 28(3):137-144.

[77] Arneberg P, Bjertness E, Storhaug K, Glennas A, Bjerkhoel F: Remaining teeth, oral dryness and dental health habits in middle-aged Norwegian rheumatoid arthritis patients. *Community dentistry and oral epidemiology*1992, 20(5):292-296.

[78] Bozkurt FY, Berker E, Akkus S, Bulut S: Relationship between interleukin-6 levels in gingival crevicular fluid and periodontal status in patients with rheumatoid arthritis and adult periodontitis. *Journal of periodontology* 2000, 71(11):1756-1760.

[79] Genco RJ, Ho AW, Grossi SG, Dunford RG, Tedesco LA: Relationship of stress, distress and inadequate coping behaviors to periodontal disease. *Journal of periodontology* 1999, 70(7):711-723.

[80] Liao F, Li Z, Wang Y, Shi B, Gong Z, Cheng X: Porphyromonas gingivalis may play an important role in the pathogenesis of periodontitis-associated rheumatoid arthritis. *Medical hypotheses* 2009, 72(6):732-735.

[81] McGraw WT, Potempa J, Farley D, Travis J: Purification, characterization, and sequence analysis of a potential virulence factor from Porphyromonas gingivalis, peptidylarginine deiminase. *Infection and immunity*1999, 67(7):3248-3256.

[82] Gargiulo AV, Jr., Robinson J, Toto PD, Gargiulo AW: Identification of rheumatoid factor in periodontal disease. *Journal of periodontology* 1982, 53(9):568-577.
[83] The J, Ebersole JL: Rheumatoid factor (RF) distribution in periodontal disease. *Journal of clinical immunology* 1991, 11(3):132-142.
[84] Rosenstein ED, Greenwald RA, Kushner LJ, Weissmann G: Hypothesis: the humoral immune response to oral bacteria provides a stimulus for the development of rheumatoid arthritis. *Inflammation* 2004, 28(6):311-318.
[85] Bonagura VR, Artandi SE, Davidson A, Randen I, Agostino N, Thompson K, Natvig JB, Morrison SL: Mapping studies reveal unique epitopes on IgG recognized by rheumatoid arthritis-derived monoclonal rheumatoid factors. *J Immunol* 1993, 151(7):3840-3852.
[86] Martin T, Crouzier R, Weber JC, Kipps TJ, Pasquali JL: Structure-function studies on a polyreactive (natural) autoantibody. Polyreactivity is dependent on somatically generated sequences in the third complementarity-determining region of the antibody heavy chain. *J Immunol* 1994, 152(12):5988-5996.
[87] Deodhar AA, Woolf AD: Bone mass measurement and bone metabolism in rheumatoid arthritis: a review. *Br J Rheumatol* 1996, 35(4):309-322.
[88] Walsh NC, Crotti TN, Goldring SR, Gravallese EM: Rheumatic diseases: the effects of inflammation on bone. *Immunological reviews* 2005, 208:228-251.
[89] Kaye EK: Bone health and oral health. *J Am Dent Assoc* 2007, 138(5):616-619.
[90] Krall EA: Osteoporosis and the risk of tooth loss. *Clin Calcium* 2006, 16(2):287-290.
[91] Taguchi A, Sanada M, Suei Y, Ohtsuka M, Nakamoto T, Lee K, Tsuda M, Ohama K, Tanimoto K, Bollen AM: Effect of estrogen use on tooth retention, oral bone height, and oral bone porosity in Japanese postmenopausal women. *Menopause* 2004, 11(5):556-562.
[92] Howell TH, Williams RC: Nonsteroidal antiinflammatory drugs as inhibitors of periodontal disease progression. *Crit Rev Oral Biol Med* 1993, 4(2):177-196.
[93] Pers JO, Saraux A, Pierre R, Youinou P: Anti-TNF-alpha immunotherapy is associated with increased gingival inflammation without clinical attachment loss in subjects with rheumatoid arthritis. *Journal of periodontology* 2008, 79(9):1645-1651.
[94] Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes care* 1997, 20(7):1183-1197.
[95] Tan WC, Tay FB, Lim LP: Diabetes as a risk factor for periodontal disease: current status and future considerations. *Annals of the Academy of Medicine, Singapore*2006, 35(8):571-581.
[96] Diagnosis and classification of diabetes mellitus. *Diabetes care* 2005, 28 Suppl 1:S37-42.
[97] Schmidt AM, Weidman E, Lalla E, Yan SD, Hori O, Cao R, Brett JG, Lamster IB: Advanced glycation endproducts (AGEs) induce oxidant stress in the gingiva: a potential mechanism underlying accelerated periodontal disease associated with diabetes. *Journal of periodontal research* 1996, 31(7):508-515.
[98] Schmidt AM, Hori O, Cao R, Yan SD, Brett J, Wautier JL, Ogawa S, Kuwabara K, Matsumoto M, Stern D: RAGE: a novel cellular receptor for advanced glycation end products. *Diabetes* 1996, 45 Suppl 3:S77-80.
[99] Mealey BL, Ocampo GL: Diabetes mellitus and periodontal disease. *Periodontol 2000* 2007, 44:127-153.

[100] Lalla RV, D'Ambrosio JA: Dental management considerations for the patient with diabetes mellitus. *Journal of the American Dental Association (1939)* 2001, 132(10):1425-1432.

[101] Mealey BL, Rose LF: Diabetes mellitus and inflammatory periodontal diseases. *Compend Contin Educ Dent* 2008, 29(7):402-408, 410, 412-403.

[102] Loos BG, Craandijk J, Hoek FJ, Wertheim-van Dillen PM, van der Velden U: Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. *Journal of periodontology* 2000, 71(10):1528-1534.

[103] D'Aiuto F, Parkar M, Andreou G, Suvan J, Brett PM, Ready D, Tonetti MS: Periodontitis and systemic inflammation: control of the local infection is associated with a reduction in serum inflammatory markers. *J Dent Res* 2004, 83(2):156-160.

[104] Grossi SG, Skrepcinski FB, DeCaro T, Robertson DC, Ho AW, Dunford RG, Genco RJ: Treatment of periodontal disease in diabetics reduces glycated hemoglobin. *Journal of periodontology* 1997, 68(8):713-719.

[105] Christgau M, Palitzsch KD, Schmalz G, Kreiner U, Frenzel S: Healing response to non-surgical periodontal therapy in patients with diabetes mellitus: clinical, microbiological, and immunologic results. *Journal of clinical periodontology* 1998, 25(2):112-124.

[106] Stewart JE, Wager KA, Friedlander AH, Zadeh HH: The effect of periodontal treatment on glycemic control in patients with type 2 diabetes mellitus. *Journal of clinical periodontology* 2001, 28(4):306-310.

[107] Westfelt E, Rylander H, Blohme G, Jonasson P, Lindhe J: The effect of periodontal therapy in diabetics. Results after 5 years. *Journal of clinical periodontology* 1996, 23(2):92-100.

[108] Grossi SG: Treatment of periodontal disease and control of diabetes: an assessment of the evidence and need for future research. *Annals of periodontology / the American Academy of Periodontology* 2001, 6(1):138-145.

[109] Teeuw WJ, Gerdes VE, Loos BG: Effect of periodontal treatment on glycemic control of diabetic patients: a systematic review and meta-analysis. *Diabetes care* 2010, 33(2):421-427.

[110] Parkin DM, Bray F, Ferlay J, Pisani P: Global cancer statistics, 2002. *CA: a cancer journal for clinicians* 2005, 55(2):74-108.

[111] Lowenfels AB, Maisonneuve P: Risk factors for pancreatic cancer. *Journal of cellular biochemistry*2005, 95(4):649-656.

[112] Stolzenberg-Solomon RZ, Graubard BI, Chari S, Limburg P, Taylor PR, Virtamo J, Albanes D: Insulin, glucose, insulin resistance, and pancreatic cancer in male smokers. *Jama* 2005, 294(22):2872-2878.

[113] Otsuki M, Tashiro M: 4. Chronic pancreatitis and pancreatic cancer, lifestyle-related diseases. *Intern Med* 2007, 46(2):109-113.

[114] Farrow B, Evers BM: Inflammation and the development of pancreatic cancer. *Surgical oncology* 2002, 10(4):153-169.

[115] Coussens LM, Werb Z: Inflammation and cancer. *Nature* 2002, 420(6917):860-867.

[116] Michaud DS, Joshipura K, GiovannucciE, Fuchs CS: A Prospective Study of Periodontal Disease and Pancreatic Cancer in US Male Health Professionals. *Journal of the National Cancer Institute* 2007, 99:171 - 175.

[117] Stolzenberg-Solomon RZ, Dodd KW, Blaser MJ, Virtamo J, Taylor PR, Albanes D: Tooth loss, pancreatic cancer, and Helicobacter pylori. *The American journal of clinical nutrition*2003, 78(1):176-181.

[118] Hujoel PP, Drangsholt M, Spiekerman C, Weiss NS: An exploration of the periodontitis-cancer association. *Annals of epidemiology*2003, 13(5):312-316.

[119] Pietinen P, Virtamo J, Taylor PR: Tooth loss is associated with increased risk of gastric non-cardia adenocarcinoma in a cohort of Finnish smokers. *Scandinavian journal of gastroenterology* 2005, 40(6):681-687.

[120] Velly AM, Franco EL, Schlecht N, Pintos J, Kowalski LP, Oliveira BV, Curado MP: Relationship between dental factors and risk of upper aerodigestive tract cancer. *Oral oncology* 1998, 34(4):284-291.

[121] Rosenquist K, Wennerberg J, Schildt EB, Bladstrom A, Goran Hansson B, Andersson G: Oral status, oral infections and some lifestyle factors as risk factors for oral and oropharyngeal squamous cell carcinoma. A population-based case-control study in southern Sweden. *Acta oto-laryngologica* 2005, 125(12):1327-1336.

[122] Irfan UM, Dawson DV, Bissada NF: Epidemiology of periodontal disease: a review and clinical perspectives. *Journal of the International Academy of Periodontology* 2001, 3(1):14-21.

[123] Garrote LF, Herrero R, Reyes RM, Vaccarella S, Anta JL, Ferbeye L, Munoz N, Franceschi S: Risk factors for cancer of the oral cavity and oro-pharynx in Cuba. *British journal of cancer* 2001, 85(1):46-54.

[124] Abnet CC, Qiao YL, Mark SD, Dong ZW, Taylor PR, Dawsey SM: Prospective study of tooth loss and incident esophageal and gastric cancers in China. *Cancer Causes Control* 2001, 12(9):847-854.

[125] Loos BG: Systemic markers of inflammation in periodontitis. *Journal of periodontology* 2005, 76(11 Suppl):2106-2115.

[126] Joshipura KJ, Wand HC, Merchant AT, Rimm EB: Periodontal disease and biomarkers related to cardiovascular disease. *Journal of dental research* 2004, 83(2):151-155.

[127] Risch HA: Etiology of pancreatic cancer, with a hypothesis concerning the role of N-nitroso compounds and excess gastric acidity. *Journal of the National Cancer Institute* 2003, 95(13):948-960.

[128] Nair J, Ohshima H, Nair UJ, Bartsch H: Endogenous formation of nitrosamines and oxidative DNA-damaging agents in tobacco users. *Critical reviews in toxicology*1996, 26(2):149-161.

[129] Shapiro KB, Hotchkiss JH, Roe DA: Quantitative relationship between oral nitrate-reducing activity and the endogenous formation of N-nitrosoamino acids in humans. *Food Chem Toxicol* 1991, 29(11):751-755.

[130] Stolzenberg-Solomon RZ, Blaser MJ, Limburg PJ, Perez-Perez G, Taylor PR, Virtamo J, Albanes D: Helicobacter pylori seropositivity as a risk factor for pancreatic cancer. *Journal of the National Cancer Institute* 2001, 93(12):937-941.

[131] de Martel C, Llosa AE, Friedman GD, Vogelman JH, Orentreich N, Stolzenberg-Solomon RZ, Parsonnet J: Helicobacter pylori infection and development of pancreatic cancer. *Cancer Epidemiol Biomarkers Prev* 2008, 17(5):1188-1194.

[132] Raderer M, Wrba F, Kornek G, Maca T, Koller DY, Weinlaender G, Hejna M, Scheithauer W: Association between Helicobacter pylori infection and pancreatic cancer. *Oncology* 1998, 55(1):16-19.

[133] Alberti KG, Zimmet P, Shaw J: The metabolic syndrome--a new worldwide definition. *Lancet* 2005, 366(9491):1059-1062.

[134] Honjo K, van Reekum R, Verhoeff NP: Alzheimer's disease and infection: do infectious agents contribute to progression of Alzheimer's disease? *Alzheimers Dement* 2009, 5(4):348-360.

[135] Lorenzo C, Okoloise M, Williams K, Stern MP, Haffner SM: The metabolic syndrome as predictor of type 2 diabetes: the San Antonio heart study. *Diabetes care* 2003, 26(11):3153-3159.

[136] Grundy SM: Metabolic syndrome: a multiplex cardiovascular risk factor. *The Journal of clinical endocrinology and metabolism*2007, 92(2):399-404.

[137] Sjoholm A, Nystrom T: Endothelial inflammation in insulin resistance. *Lancet* 2005, 365(9459):610-612.

[138] D'Aiuto F, Sabbah W, Netuveli G, Donos N, Hingorani AD, Deanfield J, Tsakos G: Association of the metabolic syndrome with severe periodontitis in a large U.S. population-based survey. *The Journal of clinical endocrinology and metabolism*2008, 93(10):3989-3994.

[139] Shimazaki Y, Saito T, Yonemoto K, Kiyohara Y, Iida M, Yamashita Y: Relationship of metabolic syndrome to periodontal disease in Japanese women: the Hisayama Study. *Journal of dental research* 2007, 86(3):271-275.

[140] Morita T, Ogawa Y, Takada K, Nishinoue N, Sasaki Y, Motohashi M, Maeno M: Association Between Periodontal Disease and Metabolic Syndrome. *J Public Health Dent* 2009.

[141] Bullon P, Morillo JM, Ramirez-Tortosa MC, Quiles JL, Newman HN, Battino M: Metabolic syndrome and periodontitis: is oxidative stress a common link? *Journal of dental research* 2009, 88(6):503-518.

[142] de la Torre JC: Alzheimer disease as a vascular disorder: nosological evidence. *Stroke; a journal of cerebral circulation*2002, 33(4):1152-1162.

[143] Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, Cooper NR, Eikelenboom P, Emmerling M, Fiebich BL *et al*: Inflammation and Alzheimer's disease. *Neurobiology of aging* 2000, 21(3):383-421.

[144] Mamelak M: Alzheimer' s disease, oxidative stress and gammahydroxybutyrate. *Neurobiology of aging* 2007, 28(9):1340-1360.

[145] Calabrese V, Scapagnini G, Giuffrida Stella AM, Bates TE, Clark JB: Mitochondrial involvement in brain function and dysfunction: relevance to aging, neurodegenerative disorders and longevity. *Neurochemical research* 2001, 26(6):739-764.

[146] Chalmers JM, Carter KD, Spencer AJ: Oral diseases and conditions in community-living older adults with and without dementia. *Spec Care Dentist* 2003, 23(1):7-17.

[147] Chalmers JM, Hodge C, Fuss JM, Spencer AJ, Carter KD: The prevalence and experience of oral diseases in Adelaide nursing home residents. *Australian dental journal* 2002, 47(2):123-130.

[148] Chapman PJ, Shaw RM: Normative dental treatment needs of Alzheimer patients. *Australian dental journal* 1991, 36(2):141-144.

[149] Ghezzi EM, Ship JA: Dementia and oral health. *Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics* 2000, 89(1):2-5.

[150] Horn VJ, Hodge WC, Treuer JP: Dental condition and weight loss in institutionalized demented patients. *Spec Care Dentist* 1994, 14(3):108-111.

[151] Nordenram G, Ryd-Kjellen E, Johansson G, Nordstrom G, Winblad B: Alzheimer's disease, oral function and nutritional status. *Gerodontology* 1996, 13(1):9-16.

[152] Ship JA: Oral health of patients with Alzheimer's disease. *Journal of the American Dental Association (1939)* 1992, 123(1):53-58.

[153] Warren JJ, Chalmers JM, Levy SM, Blanco VL, Ettinger RL: Oral health of persons with and without dementia attending a geriatric clinic. *Spec Care Dentist* 1997, 17(2):47-53.

[154] Jones JA, Lavallee N, Alman J, Sinclair C, Garcia RI: Caries incidence in patients with dementia. *Gerodontology* 1993, 10(2):76-82.

[155] Ship JA, Puckett SA: Longitudinal study on oral health in subjects with Alzheimer's disease. *Journal of the American Geriatrics Society* 1994, 42(1):57-63.

[156] Kondo K, Niino M, Shido K: A case-control study of Alzheimer's disease in Japan--significance of life-styles. *Dementia (Basel, Switzerland)* 1994, 5(6):314-326.

[157] Shimazaki Y, Soh I, Saito T, Yamashita Y, Koga T, Miyazaki H, Takehara T: Influence of dentition status on physical disability, mental impairment, and mortality in institutionalized elderly people. *Journal of dental research* 2001, 80(1):340-345.

[158] Gatz M, Mortimer JA, Fratiglioni L, Johansson B, Berg S, Reynolds CA, Pedersen NL: Potentially modifiable risk factors for dementia in identical twins. *Alzheimers Dement* 2006, 2(2):110-117.

[159] Henry RG, Wekstein DR: Providing dental care for patients diagnosed with Alzheimer's disease. *Dental clinics of North America*1997, 41(4):915-943.

[160] Ship JA, DeCarli C, Friedland RP, Baum BJ: Diminished submandibular salivary flow in dementia of the Alzheimer type. *J Gerontol* 1990, 45(2):M61-66.

[161] Miklossy J, Kasas S, Janzer RC, Ardizzoni F, Van der Loos H: Further ultrastructural evidence that spirochaetes may play a role in the aetiology of Alzheimer's disease. *Neuroreport* 1994, 5(10):1201-1204.

[162] Riviere GR, Riviere KH, Smith KS: Molecular and immunological evidence of oral Treponema in the human brain and their association with Alzheimer's disease. *Oral microbiology and immunology* 2002, 17(2):113-118.

[163] Romeo HE, Tio DL, Rahman SU, Chiappelli F, Taylor AN: The glossopharyngeal nerve as a novel pathway in immune-to-brain communication: relevance to neuroimmune surveillance of the oral cavity. *Journal of neuroimmunology* 2001, 115(1-2):91-100.

[164] Kamer AR, Craig RG, Dasanayake AP, Brys M, Glodzik-Sobanska L, de Leon MJ: Inflammation and Alzheimer's disease: possible role of periodontal diseases. *Alzheimers Dement* 2008, 4(4):242-250.

[165] Kronfol Z, Remick DG: Cytokines and the brain: implications for clinical psychiatry. *The American journal of psychiatry* 2000, 157(5):683-694.

[166] McDevitt MJ, Wang HY, Knobelman C, Newman MG, di Giovine FS, Timms J, Duff GW, Kornman KS: Interleukin-1 genetic association with periodontitis in clinical practice. *Journal of periodontology* 2000, 71(2):156-163.

[167] Grimaldi LM, Casadei VM, Ferri C, Veglia F, Licastro F, Annoni G, Biunno I, De Bellis G, Sorbi S, Mariani C *et al*: Association of early-onset Alzheimer's disease with an interleukin-1alpha gene polymorphism. *Annals of neurology* 2000, 47(3):361-365.

[168] Rebeck GW: Confirmation of the genetic association of interleukin-1A with early onset sporadic Alzheimer's disease. *Neuroscience letters* 2000, 293(1):75-77.

[169] Yucesoy B, Peila R, White LR, Wu KM, Johnson VJ, Kashon ML, Luster MI, Launer LJ: Association of interleukin-1 gene polymorphisms with dementia in a community-based sample: the Honolulu-Asia Aging Study. *Neurobiology of aging* 2006, 27(2):211-217.

[170] Mrak RE, Griffin WS: Interleukin-1, neuroinflammation, and Alzheimer's disease. *Neurobiology of aging* 2001, 22(6):903-908.

[171] K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 2002, 39(2 Suppl 1):S1-266.

[172] Fisher MA, Taylor GW, Shelton BJ, Jamerson KA, Rahman M, Ojo AO, Sehgal AR: Periodontal disease and other nontraditional risk factors for CKD. *Am J Kidney Dis* 2008, 51(1):45-52.

[173] Davidovich E, Schwarz Z, Davidovitch M, Eidelman E, Bimstein E: Oral findings and periodontal status in children, adolescents and young adults suffering from renal failure. *Journal of clinical periodontology* 2005, 32(10):1076-1082.

[174] Proctor R, Kumar N, Stein A, Moles D, Porter S: Oral and dental aspects of chronic renal failure. *Journal of dental research* 2005, 84(3):199-208.

[175] Ioannidou E, Kao D, Chang N, Burleson J, Dongari-Bagtzoglou A: Elevated serum interleukin-6 (IL-6) in solid-organ transplant recipients is positively associated with tissue destruction and IL-6 gene expression in the periodontium. *Journal of periodontology* 2006, 77(11):1871-1878.

[176] Spolidorio LC, Spolidorio DM, Massucato EM, Neppelenbroek KH, Campanha NH, Sanches MH: Oral health in renal transplant recipients administered cyclosporin A or tacrolimus. *Oral diseases* 2006, 12(3):309-314.

[177] 1Tonelli M, Sacks F, Pfeffer M, Jhangri GS, Curhan G: Biomarkers of inflammation and progression of chronic kidney disease. *Kidney international* 2005, 68(1):237-245.

[178] Consensus Conference:From the National Institutes of Health. Osteoporosis prevention, diagnosis, and therapy. . *J Am Med Assoc* 2001, 285:785-795.

[179] Geurs NC: Osteoporosis and periodontal disease. *Periodontol 2000* 2007, 44:29-43.

[180] Lerner UH: Inflammation-induced bone remodeling in periodontal disease and the influence of post-menopausal osteoporosis. *Journal of dental research* 2006, 85(7):596-607.

[181] Jeffcoat MK, Lewis CE, Reddy MS, Wang CY, Redford M: Post-menopausal bone loss and its relationship to oral bone loss. *Periodontol 2000* 2000, 23:94-102.

[182] Wactawski-Wende J, Hausmann E, Hovey K, Trevisan M, Grossi S, Genco RJ: The association between osteoporosis and alveolar crestal height in postmenopausal women. *Journal of periodontology* 2005, 76(11 Suppl):2116-2124.

[183] Brennan RM, Genco RJ, Hovey KM, Trevisan M, Wactawski-Wende J: Clinical attachment loss, systemic bone density, and subgingival calculus in postmenopausal women. *Journal of periodontology* 2007, 78(11):2104-2111.

[184] Friedewald VE, Kornman KS, Beck JD, Genco R, Goldfine A, Libby P, Offenbacher S, Ridker PM, Van Dyke TE, Roberts WC: The American Journal of Cardiology and Journal of Periodontology editors' consensus: periodontitis and atherosclerotic cardiovascular disease. *Journal of periodontology* 2009, 80(7):1021-1032.

[185] Wilson PW, Castelli WP, Kannel WB: Coronary risk prediction in adults (the Framingham Heart Study). *Am J Cardiol* 1987, 59(14):91G-94G.

[186] Matthews DC: Periodontal medicine: a new paradigm. *J Can Dent Assoc* 2000, 66(9):488-491.

[187] Humphrey LL, Fu R, Buckley DI, Freeman M, Helfand M: Periodontal disease and coronary heart disease incidence: a systematic review and meta-analysis. *Journal of general internal medicine* 2008, 23(12):2079-2086.

[188] Paraskevas S, Huizinga JD, Loos BG: A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. *Journal of clinical periodontology* 2008, 35(4):277-290.

[189] Herzberg MC, Weyer MW: Dental plaque, platelets, and cardiovascular diseases. *Ann Periodontol* 1998, 3(1):151-160.

[190] Ross R: Atherosclerosis--an inflammatory disease. *N Engl J Med* 1999, 340(2):115-126.

[191] Beck JD, Elter JR, Heiss G, Couper D, Mauriello SM, Offenbacher S: Relationship of periodontal disease to carotid artery intima-media wall thickness: the atherosclerosis risk in communities (ARIC) study. *Arterioscler Thromb Vasc Biol* 2001, 21(11):1816-1822.

[192] Guiglia R, Lo Russo L, Coccia E, Di Liberto C, D'Angelo M, Indovina G, Lo Muzio L, Campisi G: The association between periodontal diseases and cardiovascular diseases: a narrative review. *Panminerva medica* 2008, 50(4):327-337.

[193] Tonetti MS, D'Aiuto F, Nibali L, Donald A, Storry C, Parkar M, Suvan J, Hingorani AD, Vallance P, Deanfield J: Treatment of periodontitis and endothelial function. *The New England journal of medicine* 2007, 356(9):911-920.

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Chapter XI

Obesity Revised

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Obesity, diabetes and oral diseases (dental cariesand periodontal diseases), largely preventable chronic diseases, are described as global pandemic due their distribution and severe consequences. WHO has called for a global action for prevention and promotion of these diseases as a vital investment in urgent need.

Diabetes and obesity, showing an increasing trend, lead to disabilities and negatively impacts on the quality of life through life course along with oral diseases. WHO projects that the prevalence of diabetes and deaths/year attrituble to diabetes complications will double worldwide by 2030. Globally, more than 1 billion adults are overweight; almost 300 million of them are clinically obese. Being obese/overweight raises steeply the likelihood of developing DM2. Approximately 85% of people with diabetes are DM2, and of these 90% are obese or overweight. Obesity increases the likelihood of periodontitis which is one of the most common chronic diseases worldwide, described as pandemic, and closely related to DM2. Promoting good oral health is significantly essential for prevention and reducing the negative consequences of periodontal diseases, DM2 and obesity, and to maintain good health, as proposed by European health goals by WHO.

Successful maintenance of a high glycemic control, DM2 management and obesity, and good oral healthdepends on adherence to the regime of daily treatment and self-care practices. However, many patients find themselves unable to follow recommended lifestyles (a healthy diet, physical exercise, no smoking, twice daily toothbrushing), which makes them more prone to diabetes-related complications, poor oral health, periodontal health in particular, and obesity, therefore leading a poor quality of life.

WHO, International Diabetes Federation (IDF) and The World Dental Federation (FDI) has declared that there is a need to adopt a common-risk factor approach for oral and general health promotion; a need for interventions integrating oral health into chronic disease management; behavioural interventions are highly recommended by WHO. Health Coaching

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(HC), a health promotion tool, is a new innovative `behavioural intervention that facilitates individuals in establishing and attaining health promoting goals in order to change lifestyle-related behaviours, with the intend of reducing health risks, improving self-management of chronic-conditions, and increasing health-related quality of life`. HC is demonstrated among the most effective behavioural techniquesthat contributes to positive behavioural outcomes (smoking cessation, obesity and diabetes management) but it has not been used as a holistic intervention for oral health and DM2 and obesity.

The aim of this chapter is to present background, significance, the methodology and the framework for a new structured oral health coaching intervention-first time to our knowledge-for DM2 patients considering the common biomedical and lifestyle factors interrelated with obesity, DM2, and oral health.

Prevalence of Obesity

Overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health (1). The prevalence of overweight and obesity is commonly assessed by using body mass index (BMI), defined as the weight in kilograms divided by the square of the height in metres (kg/m^2). Globally, a BMI over a 25 kg/m^2 is defined as overweight, and a BMI of over 30 kg/m2 as obese among adults (1).

Overweight and obesity, measured according to BMI criteria, are major risk factors for a number of chronic diseases, including diabetes, cardiovascular diseases and cancer. However, excess deposition of fat in the abdominal region is associated strongly with the metabolic disturbances, underlying many of the obesity-related conditions and many authors recommend measures of abdominal adiposity as additional preferred measures (2-5). One those measures, waist circumference is a convenient and simple measure which is unrelated to height, correlates closely with BMI and the ratio of waist-to-hip circumference, and is an approximate index of intra-abdominal fat mass and total body fat. Furthermore, changes in waist circumference reflect changes in risk factors for cardiovascular disease and other forms of chronic diseases, even though the risks seem to vary in different populations (2).

Today, there is a worldwide large shift in communities towards more energy-dense, nutrient-poor foods - diets with a higher proportion of fats, saturated fats and sugars-, and at the same time towards less physically active work and social life, increasing use of automated transport, eating outside, technology in the home, and more passive leisure pursuits. Therefore, the effectiveness over the long term of most dietary strategies for weight lossto battle against obesityremains uncertain unless accompanied by changes in behaviour affecting physical activity and food habits (1). Besides, health promotion strategies which focus on education about weight loss and physical activity, neglecting behavioural and social determinants of obesity, lead to short term success. Community-based strategies to promote health by taking into consideration the behavioural and social determinants are highly suggested by international health organisations, such as WHO (6).

Prevalence of Diabetes

"Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces. Hyperglycaemia, or raised blood sugar, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels.

- **Type 1 diabetes** (previously known as insulin-dependent, juvenile or childhood-onset) is characterized by deficient insulin production and requires daily administration of insulin.
- Symptoms include excessive excretion of urine (polyuria), thirst (polydipsia), constant hunger, weight loss, vision changes and fatigue. These symptoms may occur suddenly.
- **Type 2 diabetes** (formerly called non-insulin-dependent or adult-onset) results from the body's ineffective use of insulin. Type 2 diabetes comprises 90% of people with diabetes around the world, and is largely the result of excess body weight and physical inactivity.

Symptoms may be similar to those of Type 1 diabetes, but are often less marked, thus the disease may be diagnosed several years after onset, once complications have already arisen.

Until recently, this type of diabetes was seen only in adults but it is now also occurring in children and it is growing worldwide" (7).

There are almost 200 million people with diabetesat present, and 3,2 million deaths/yearare attrituble to diabetes complications and these will doubleworldwide by 2030 (7-10).Being obese or overweightraises steeply the likelihood of developing DM2. Approximately 85% of people with diabetes are DM2, and of these 90% are obese or overweight (8).

Environmental factors play a key role in the development of type 2 diabetes. Globalization and industrialization are the underlying cause for the excess of high-density, low-nutrient food and drink throughout the world, and an increasing tendency for children to be sedentary and unfit, thus leading to a global epidemic of obesity as a major risk factor for type 2 diabetes (10, 11).

Lifestyle measures have been shown to be effective in preventing or delaying the onset of type 2 diabetes, thusthere is need for:

- achievement and maintenance of a healthy body weight;
- physical activity – at least 30 minutes of regular, moderate-intensity activity on most days. More activity is required for weight control;
- a healthy diet of between three and five servings of fruit and vegetables a day and reduce sugar and saturated fats intake;
- avoidance of tobacco use – smoking increases the risk of cardiovascular diseases (10).

In brief, type 2 diabetes is a lifestyle disease; thus is essential that the patient acquires the knowledge and skills necessary to be able to achieve a satisfactory level of self-care.The

treatment of Type 2 diabetes is initially based on lifestyle changes, therefore in its treatment. Non-pharmacological treatment, behavioural approaches, will often entail such great lifestyle changes for the patient that they are very difficult to carry out (12).

Prevalence of Oral Diseases

Despite great achievements in the oral health of populations globally, oral diseases are among the major global health problems, particularly among underprivileged groups in developed and developing countries, affecting a vast majority of individuals-. Dental caries and periodontal diseases are described as global pandemic due their distribution and severe consequences (13). This may be interpreted as a threat for global health because oral health is an integral part of general health, namely general well-being, (Figure 1)(14). WHO has defined health as "the state of complete physical, mental and social well-being and the absence of disease or infirmity" (15).Most oral diseases contribute negatively to the health as they share the common environmental and behavioral risk factors with chronic diseases(16, 17) such that diabetes, obesity and oral diseases have common lifestyle risk factors (poor dietary habits, a sugar-rich diet, smoking) (18-22).

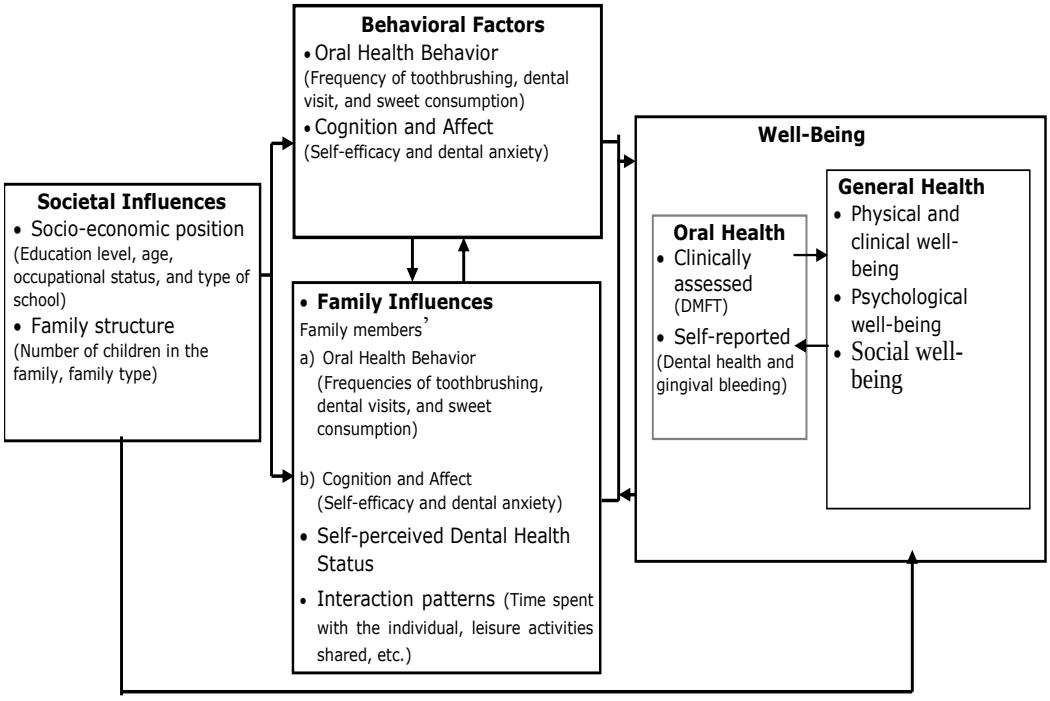

Figure 1. The interrelation between oral and well-being (modified from "Oral Health Promotion Model for Preadolescents" by Cinar, 2008).

Far beyond, current scientific evidence provides evidence that diabetes, obesity and oral diseases have common biologic mechanisms (23-29). There seems to be a bidirectional relationship between diabetes and oral health: Poor oral health negatively contributes to glycemic control whereas poor type 2 diabetes (DM2) management negatively affects oral

health (29). Thus, they lead to poor systemic health conditions (30). Obesity is a triggering risk factor both for DM2 and periodontal diseases (23-25).

For better health promotion and prevention of diabetes type 2 and obesity, there is need to assess the structure of oral diseases beyond the clinical aspects.Dental caries and periodontal diseases can be seen as behavioral and social diseases (14, 31-33), like diabetes type 2 and obesity. Dental caries is the outcome of the disease process, which includes pathological factors (acidogenic bacteria, sugar), and which starts under the influence of initiative factors such as social factors including attitudes and behaviors (34). Periodontal disease, like other chronic diseases, is socially patterned, composed of the psychosocial factors that lead to change in the oral environment and in behavioral responses of the host, such as poor oral hygiene and smoking (33). The quantitative and qualitative composition of the resident oral microflora is dictated by the oral environment, whose response to the bacteria is affected by psychosocial factors and the behaviours of the individual (33).

Therefore, successful maintenance of a high glycemic control, DM2 management and obesity, and good oral healthdepends on the adherence to the regime of daily treatment and self-care practices (14, 35-37). However, many patients find or feel themselves unable to follow recommended lifestyles (a healthy diet, physical exercise, no smoking, medications,twice daily toothbrushing), which makes them more prone to diabetes-related complications, poor oral health and obesity, therefore leading a poor quality of life.

WHO (13), International Diabetes Federation (IDF)(38), The World Dental Federation (FDI)(39)and Council of European Dentists (40),American Dental Association (41) underline a need to adopt a common-risk factor approach (42) for oral and general healthpromotion; a need for interventions integrating oral healthinto chronic disease management. WHO highly recommends behavioural interventions to meet this need (43).

Health Coaching (HC) a patient-centred and motivational interviewing-based approach (Table 1)(44) is increasingly being incorporated into health management programs. HC, a health promotion tool, is an innovative`behavioural intervention that facilitates individuals in establishing and attaining health promoting goals in order to change lifestyle-related behaviours, with the intend of reducing health risks, improving self-management of chronic-conditions, and increasing health-related quality of life` (45). HC is demonstrated as the most effective behavioural technique causally associated with positive behavioural outcomes (smokingcessation, obesity and diabetes management) (46- 52) but it has not been used as a holistic interventionfor oral health and DM2 and obesity.

An international prospective study was designed to assess the impact of an oral health-coaching based intervention on oral and general health (DM2, obesity, quality of life)- to our knowledge for the first time- among adults in Turkey and Denmark by using subjective (self-reports) and objective (clinical) measurements by AB Cinar as the principal investigator. The study, compromising two 3-months intervention and a 6-month follow-up, will start in September 2010 in Turkey.

At initial stage all the patients recruited from outpatients clinics of the participating hospitals will be examined for clinical examination for general health (HA1C, fasting blood glucose, post-prandial glucose, BMI, body-fat ratio) and oral health(dental cariesand periodontal diseases). On the day of clinical examinations, questionnaires that include well-known diabetes and oral health-related scales will be distributed and collected back. Following this event, all patients will be invited to a short seminar about oral diseases and its relation with diabetes and obesity. Then, the patients will be randomly allocated either to

intervention (coaching group) or control (formal training), groups. Coaching sessions will be performed in three formats (group, individual and telephone coaching) as two 3-months interventions with a month break interval between. The control group takes formal education focused on oral health and its relation with diabetes, obesity and quality of life. At the end of two 3-months intervention and the 6-months follow-up, clinical and biomedical, and psycho-social and behavioral outcomes (Figure 2) will be assessed by clinical examinations and self-assessed questionnaires.

Table1. Comparison of traditional health education and the ideal health coaching approach*

Aspect	Ideal Health Coaching Approach	Traditional Health Education
Orientation	Patient-oriented	Task-oriented
Most common techniques used	Expression of empathy, rolling with resistance, supporting self-efficacy, developing discrepancy	Advice-giving, information sharing, personal testimonies
Approach to disease management	Whole-person approach, where behaviours are prioritized for maximum impact on overall health	Management of the disease and its complications
Behaviour change models used	Health Belief Model, Self-Perception Theory, Social Cognitive Theory, Value Theory, Stages of change, Implementations Intentions Model	Infrequently used; stages of change most often reported
Technique used	Motivational Interviewing	None
Decision-making process about which behaviours to change/adopt	Collaborative effort between health care professional and the patient Health careprofessionals are directive in guiding the patients towards exploring risk reduction and facilitating movement through stages of change	Health care professional decides what is best for the patient using evidence-based practice guidelines
Treatment Plan	A fully developed plan of action on the participant's intentions	Advice by health care professional to adhere the prescribed treatment guidelines
Orientation	Patient-oriented	Task-oriented

* Modified from Butterworth et al. (2007).

The scope of the research falls within joint declaration of FDI and IDF (38): a) to include prevention of oral disease and promotion of oral health as an essential component of diabetes management; b) to initiate and support research leading to evidence-based treatment strategies to improve health and oral health of people with diabetes; c) to introduce screening for diabetes in the dental office among high-risk populations;d) to improve knowledge about the reciprocal link between diabetes and oral health among all stakeholders, health professionals, people with diabetes, the public and policy makers.

There are many researches in the field of promotion and prevention of diabetes and oral health. However, the study is unique there is no research-to our knowledge- that there is not any study -to our knowledge- considering oral health promotion for DM2 that assesses both oral health and diabetes-related subjective and clinical outcomes. In addition, the study provides an opportunity to assess if the concept of oral health becomes part of global health culture, as already indicated by an earlier research (53).

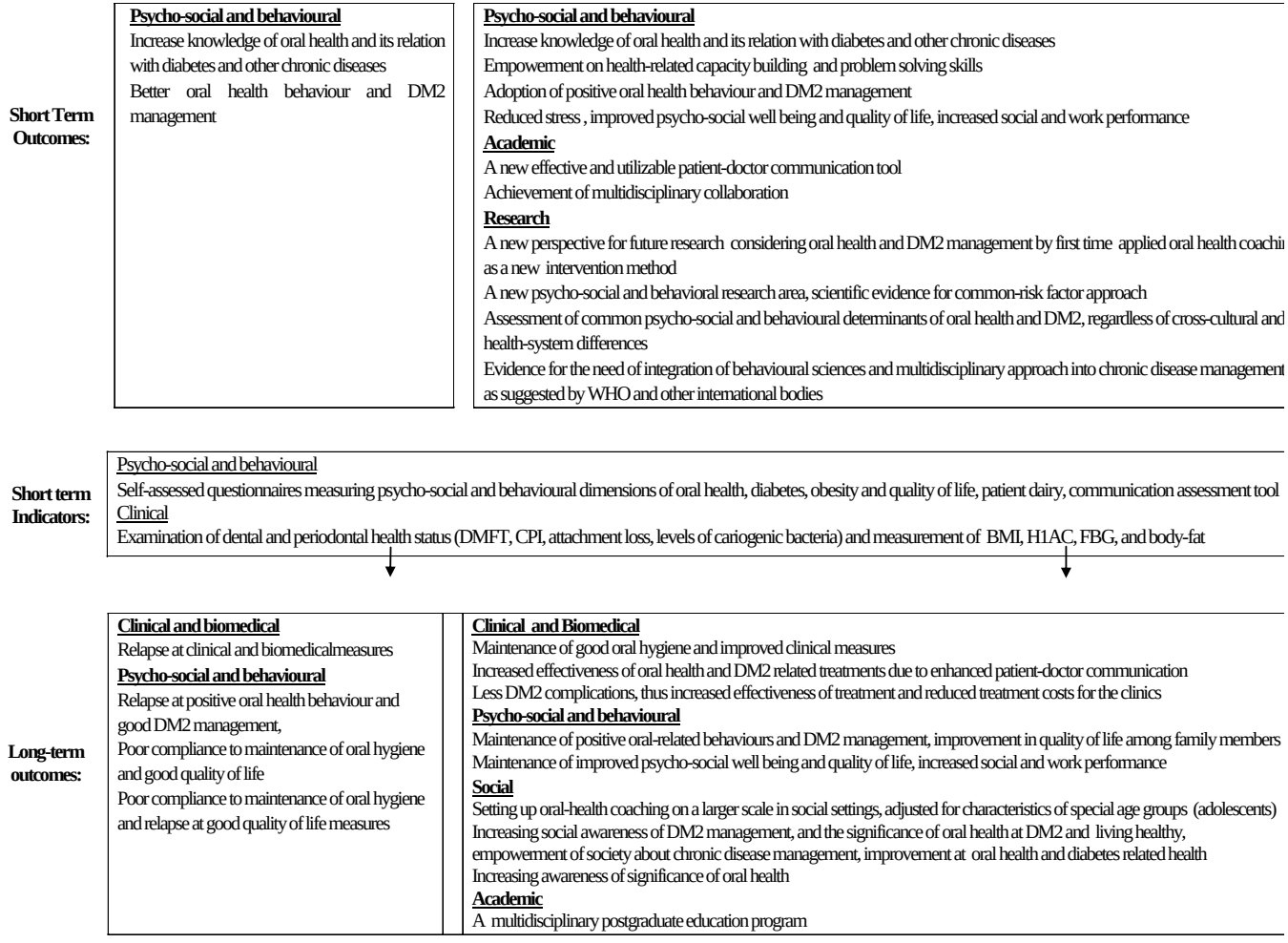

Figure 2. The short- and long-term expected outcomes of the study

References

[1] *http://www.who.int/dietphysicalactivity/publications/facts/obesity*

[2] Van Itallie TB. Waist circumference: a useful index in clinical care and health promotion. *Nutr Rev* 1998; 56: 300-302.

[3] DespreÂs J-P. Body fat distribution and cardiovascular risk: importance of visceral fat. *Medicographia* 1994; 16: 11-15.

[4] Wei M, Gaskill SP, Haffner SM, Stern MP. Waist circumference as the best predictor of noninsulin dependent diabetes mellitus (NIDDM) compared to body mass index, waist=hip ratio and other anthropometric measurements in Mexican Americans: a 7-year prospective study. *Obes Res* 1997; 5:16 -23.

[5] *http://www.who.int/nutrition*

[6] *www.wpro.who.int/.../07_Chapter2Socialdeterminantsofhealth.pdf*

[7] *http://www.who.int/diabetes*

[8] The global burden of chronic diseases. WHO, 2010. *http://www.who.int/nutrition/topics/2_background/en/index.html*

[9] Diabetes. WHO, 2010. *http://www.who.int/mediacentre/factsheets/fs312/en/*

[10] 10.Global Strategy on Diet, Physical Activity and Health: Facts related to diabetes. WHO, 2010. *http://www.who.int/dietphysicalactivity/publications/facts/*

[11] *http://www.idf.org/position-statement-type-2-diabetes*

[12] *Type 2 Diabetes: Health Technology Assessment of screening, diagnosis and treatment.* Danish Health Technology Assessment 2005; 7:

[13] Petersen PE. The World Oral Health Report 2003: continuous improvement of oral health in the 21st century-the approach of the WHO Global Oral Health Programme. *Community Dent Oral Epidemiol* 2003; 31 Suppl 1:3-23.

[14] Cinar AB. Preadolescents and Their Mothers as Oral Health-Promoting Actors: Non-biologic Determinants of Oral Health among Turkish and Finnish Preadolescents. *Doctorate Thesis*. Helsinki: University of Helsinki 2008.

[15] WHO. Constitution. New York: WHO; 1946. In Downie RS, Tannahill C, Tannahill A. *Health Promotion: Models and Values.* Oxford: Oxford University Press; 1996. p.9.

[16] Petersen PE, BourgeoisD, Ogawa H, Estupinan-Day S, Ndiaye C. The globalburden of oral diseases and risks to oral health. *Bull World Health* 2005; 83: 661-9.

[17] Petersen PE, Estupinan-Day S, Ndiaye C. WHO's action for continuous improvement in oral health. *Bull World Health* 2005; 83: 641-720.

[18] Marshall TA, Eichenberger-Gilmore JM, Broffitt BA, et al. Dental caries and childhood obesity: roles of diet and socio-economic status. *Community Dent Oral Epidemiol* 2007; 35:449-458.

[19] Alm A, Fahreus C, Wendt LK, et al. Body adiposity status in teenagers and snacking habits in early childhood in relation to proximal caries at 15 years of age. *Int J Paediatr Dent*2008; 18: 189-186.

[20] WHO. Diet nutrition and the prevention of chronic diseases. Report of the joint WHO/FAO expert consultation, *WHO Technical Report Series*, No.916 (TRS 916). WHO: Geneva, 2003.

[21] Cinar AB, Murtomaa H. Clustering of Obesity and Dental Health with Life-Style Factors among Turkish and Finnish Pre-adolescents. *Obesity Facts* 2008; 1:196-202.

[22] Cinar AB, Murtomaa H. Interrelation between obesity, oral health and life-style factors among Turkish school children. *Clin Oral Investig*2010 Jan. [Epub ahead of print]

[23] Pischon N, Heng N, Bernimoulin JP, et al.Obesity, inflammation, and periodontal disease.*J Dent Res* 2007;86: 400-9.

[24] Al-Zahrani MS. Obesity and Periodontal Disease in Young, Middle-Aged, and Older Adults *J Periodontol*. 2003;74: 610-615.

[25] Genco RJ, Grossi SG, Ho A, et al. A proposed model linking inflammation to obesity, diabetes, and periodontal infections.*J Periodontol* 2005; 76(11 Suppl): 2075-2084.

[26] Lundin M, Yucel-Lindberg T, Dahllöf G,et al.Correlation between TNFalpha in gingival crevicular fluid and body mass index in obese subjects.*Acta Odontol Scand* 2004;62(5):273-277.

[27] Ritchie CS, Kinane DF. Nutrition, inflammation*Nutrition* 2003; 19:475-476.

[28] Nishimura F, Soga Y, Iwamoto Y, et al. Periodontal disease as part of the insulin*J Int Acad Periodontol*. 2005;7: 16-20.

[29] Nishimura F, Kono T, Fujimoto C, et al. Negative effects of chronic inflammatory periodontal disease on diabetes mellitus.*J Int Acad Periodontol* 2000;2 :49-55.

[30] Santacroce L, Carlaio RG, Bottalico L. Does it Make Sense that Diabetes is Reciprocally Associated with Periodontal Disease?*Endocr Metab Immune Disord Drug* Targets, 2010. [Epub ahead of print]

[31] Fejerskov O, Kidd E (eds). *Dental Caries: The Disease and its Clinical Management*. USA: Blackwell Munksgaard; 2003. p. 165-77.

[32] Kuusela S, Honkala E, Kannas L, Tynjälä J, Wold B.Oral hygiene habits of 11-year-old schoolchildren in 22 European countries and Canada in 1993/1994. *J Dent Res* 1997; 76: 1602-9.

[33] Sheiham A, Netuveli GS. Periodontal diseases in Europe. *Periodontol* 2000 2002; 29: 104-21.

[34] Eriksen HM, Dimitrov V. Ecology of oral health: a complexity perspective. *Eur J Oral Sci* 2003; 111: 285-90.

[35] Funnell MM. Peer-based behavioural strategies to improve chronic disease self-management and clinical outcomes: evidence, logistics, evaluation considerations and needs for future research. *Family Practice 2009*. doi:10.1093/fampra/cmp027.

[36] BhuyanKK. Health promotion through self-care and community participation: Elements of a proposed programme in the developing countries. *BMC Public Health* 2004. doi:10.1186/1471-2458-4-11

[37] Minet L, Møller S, Vach W, et al.Mediating the effect of self-care management intervention in type 2 diabetes: A meta-analysis of 47 randomised controlled trials.*Patient Educ Couns* 2009. doi:10.1016/j.pec.2009.09.33

[38] IDF. Diabetes and oral health**.** Available at: *http://www.idf.org/diabetes*.

[39] FDI. People with diabetes need to pay special attention to oral health, 2007. *http://www.fdiworldental.org/content/people-diabetes-need-pay-special-attention-oral-health*

[40] Council of European Dentists. *http://ec.europa.eu/health/ph_overview/strategy/docs/R-031.pdf*

[41] American Dental Association. *http://ebd.ada.org/*

[42] Sheiham A, Watt RG. The common risk*Community Dent Oral Epidemiol* 2000; 28:399-406.

[43] WHO. Facts related to chronic diseases. *http://www.who.int/dietphysicalactivity/publications/facts/chronic/en/*

[44] Cinar AB, Murtomaa H. *Health Coaching as an Oral Health Education Tool*. Poster presentation. ADEE Congress, Helsinki-Finland, 26-29 August 2009.

[45] Butterworth SW, Linden A, McClay W. Health coaching as an intervention in health management programs.*Dis Manage Health Outcomes* 2007; 15: 299-307.

[46] Lancaster T, Stead LF. Individualbehavioural counselling for smoking cessation. *Cochrane Database Syst Rev*2005: 2.

[47] Stevens VJ, Glasgow RE, Toobert DJ, et al. One-year results from a brief, computer-assisted intervention to decrease consumption of fat and increase consumption of fruits and vegetables.*Prev Med* 2003; 3: 594-600.

[48] Klesges RC, Kumanyika SK, Murray DM et al.Child- and parent-targeted interventions: the Memphis GEMS pilot study.*Ethn Dis.* 2003; 13(1 Suppl 1):S40-53.

[49] Bacon L, Stern JS, Van Loan MD, Keim NL. Size acceptance and intuitive eating improve health for obese, female chronic dieters.*J Am Diet Assoc* 2005; 105: 929-936.

[50] Sarvestani RS, Jamalfard MH, Kargar M, Kaveh MH, Tabatabaee HR. Effect of dietary behaviour modification on anthropometric indices and eating behaviour in obese adolescent girls.*J Adv Nurs* 2009; 65: 1670-5.

[51] Whittemore R, D'Eramo Melkus G, Grey M. Metabolic*J Clin Nurs* 2005;14:195-203.

[52] Whittemore R, Melkus GD, Sullivan A, Grey M. A nurse-coaching intervention for women with type 2 diabetes. *Diabetes Educ* 2004; 30: 795-804.

[53] Cinar AB, Tseveenjav B, Murtomaa H.Oral health-related self-efficacy beliefs and toothbrushing: Finnish and Turkish pre-adolescents' and their mothers' responses.*Oral Health Prev Dent*2009;7: 173-81.

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Chapter XII

Invasion of Host Cells by *Porphyromonas Gingivalis* in Polymicrobial Infection

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Abstract

Periodontitis is one of the predominant polymicrobial infections of humans. Since periodontitis results from complex interactions of multiple microorganisms, it is important to investigate interactions between different periodontal bacteria and host cells. *Porphyromonas gingivalis*, a gram-negative anaerobe, is a major colonizer of gingival tissues and has been etiologically implicated in periodontal as well as cardiovascular diseases. Cellular invasion by periodontal pathogens including *P. gingivalis* has been proposed as a possible virulence factor, affording protection from the host immune responses and contributing to tissue damage. In recent periodontal research, polymicrobial infection models have been used to study host response profiles. However, data on the potential of host cell invasion by periodontal pathogens in polymicrobial infection are scarce. We investigated the ability of periodontal pathogens to modulate invasion of human gingival epithelial cells and aortic endothelial cells by *P. gingivalis*. Among the pathogens, *Fusobacterium nucleatum* was shown to significantly enhance the *P. gingivalis* invasion. We describe the complex interaction between periodontopathogens and host cells, with a particular focus on the co-infection by *P. gingivalis* and *F. nucleatum*.

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Introduction

Porphyromonas gingivalis, a gram-negative anaerobe, is a major colonizer of gingival tissues and has been etiologically implicated in various forms of periodontitis [1]. Epidemiological studies have demonstrated a positive association between periodontitis and cardiovascular diseases [2]. It has been suggested that infection by periodontal pathogens may be involved in the development of the atherosclerotic plaque[3-5]. Periodontal pathogens including *P. gingivalis*have been detected in atherosclerotic plaques in humans using PCR techniques [6-8].

P. gingivalis elicits a proatherogenic response in endothelial cells in the form of increased leukocyte adhesion with concomitant up-regulation of adhesion molecules,heightened production of proinflammatory cytokines and chemokines, as well as an induction of prothrombotic properties[9, 10]. These effects on endothelial cells cannot be attributed to a sole effect of stimulation by bacterial cell-surface components, but may require the invasion of hostcells by viable bacteria[11, 12].

Bacterial colonization of soft-tissue structures is central to periodontal disease pathogenesis since microbes and their products gain access to the subepithelial connective tissue from the sulcular region of gingiva, where the pathophysiological process of periodontal disease is initiated [13]. To establish a chronic infection in a hostile host environment, it is imperative that pathogens find ways to evade or subvert host defense mechanisms [14]. Toward this end, many pathogenic microorganisms have evolved abilities to invade host cells, replicate intracellularly and spread from cell to cell. Host cell invasion by *P.gingivalis* has been proposed as a possible mechanism of pathogenesis in periodontal and cardiovascular diseases[15, 16].*P. gingivalis* can disseminate intravascularly during the transient bacteremias that result from mastication or oral hygiene procedures[17, 18]. Under these circumstances, *P. gingivalis* will be in contact with the endothelial cells that line the vessels of the circulatory system, and *P. gingivalis* has been shown to adhere to and invade endothelial cells [8, 18].

Periodontitis is one of the most prevalent polymicrobial infections of humans[19].Given that periodontitis results from complex interactions of multiple microorganisms, it is essential to investigate interactions between different periodontal bacteria and host cells. Bacterial species in one of the fivesubgingival plaquecomplexes referred to as “red complex” are commonly associated with periodontal lesions[20]. The red complex bacteria such as *P. gingivalis* are often detected in the presence of bacterial complex referred to as “orange complex” comprising, e.g.,*Fusobacterium nucleatum*[20, 21].F. nucleate, a gram-negative fusiform anaerobe, coaggregates with microbial species in the oral cavity, playing a critical role in periodontal biofilm formation. Antagonistic and synergistic physiologic mechanisms, as well as environmental selection are thought to be involved in such relationships[21].*F. nucleatum* initially adheres to early colonizers, including gram-positive cocci, and enhances the adherence of other periodontopathic bacteria including*P.gingivalis*[22]. In polymicrobial infections by bacterial enteropathogens, it has been shown that the ability of *Campylobacter jejuni* to invade cultured epithelial cells is significantly enhanced by the presence of other enteropathogens as coinfectants[23]. Whether similar interaction occurs in periodontopathogens is unknown. Data on the potential of *P.gingivalis* invasion into host cells in polymicrobial infection are scarce; we therefore investigated the capacity of

P.gingivalis to invade human gingival epithelial and aortic endothelial cells in co-infection with several major periodontal pathogens [24, 25].

Methods

Bacterial Strains and Growth Conditions

The following bacterial strains were used; *P. gingivalis* ATCC 33277, *P. gingivalis*W83 (ATCC BAA-308) (American Type Culture Collection, Rockville, MD, USA), *F. nucleatum*ATCC 25586, #20, TDC100 (a clinical isolate and working strain in our laboratory) [26], *Aggregatibacteractinomycetemcomitans* JP2, Y4, 310a (kindly provided by Dr. H. Ohta, Ibaraki University, Japan), *Treponema denticola* ATCC35405, *Tannerella forsythia* ATCC43037, *Prevotella intermedia* ATCC 25611. *P. gingivalis, F. nucleatum, A. actinomycetemcomitans* and *P. intermedia*were grown in brainheart infusion (BHI) broth (Becton Dickinson, Sparks, MD, USA) supplemented with 0.5 % of yeast extract, hemin (5 µg/ml) and menadione (0.5 µg/ml). *T. denticola* was grown in TYGVS medium as described previously [27]. *T. forsythia* was grown in BHI broth supplemented with 5% heat-inactivated fetal calf serum and 0.001% N-acetyl muramic acid. The bacterial cultures were grown to mid-log phase (range at OD660nm of 0.6-1.0) at 37 °C underanaerobic conditions. Non invasive*Escherichia coli*strains were used as a control.

Heat-killed *F. nucleatum* were prepared by heating the bacteria at 80 °C for 10 min. Methanol-fixed *F. nucleatum* were prepared by fixing the bacteria in 99% methanol for 1h at room temperature (RT). The absence of bacterial growth after the treatment was confirmed by plating samples on blood agar plates.

Cells and Culture Conditions

An established human gingival epithelial cell line, Ca9-22, was purchased from Health Science Research Resources Bank (Osaka, Japan). The Ca9-22 cells were maintained in Eagle's minimal essential medium (MEM) supplemented with glutamine (0.6 mg/ml), heat-inactivated 10 % fetal calf serum, and gentamicin (10 µg/ml) / amphotericin B (0.25 µg/ml) (Cascade Biologics, Portland, OR, USA) at 37 °C in 5 % CO_2 in humidified air.

Human aorta endothelial cells (HAEC) were supplied by Kurabo Inc. (Osaka, Japan) and maintained in HuMedia-EG2 (Kurabo) under an atmosphere of 5% CO_2 and 95 % air at 37 °C. Cells from passages 4 through 9 were tested for viability and morphology prior to seeding in appropriate tissue cultureplates and allowed to reachnear-confluency before assay.

Monomicrobial Infection

Invasion of bacteria was quantitated by a standard antibiotic protection assay as described previously [15]. Briefly, epithelial cells were seeded in 12-well flat-bottom culture plates at a cell density of 2.0×10^5 cells per well. Prior to infection, the cells were washed twice with

phosphate-buffered saline (PBS, pH 7.4) and incubated further 2 h in MEM without antibiotics. The multiplicity of infection (MOI) was calculated based on the number of cells per well at confluence. The bacteria were harvested by centrifugation, washed with PBS, and resuspended in MEM at desired concentrations. The bacterial suspensions were added to confluent Ca9-22 monolayers and incubated at 37°C in 5 % CO_2 for 2h. After incubation, unattached bacteria were removed following washing of the monolayers 3 times with PBS. External adherent cells were then killed by incubating the infected monolayers with MEM containing 200 µg/ml of metronizazole and 300 µg/mlof gentamicinfor 1 h. After exposure to antibiotic, monolayers were washed twice with PBS, and lysed in 1 ml of sterile distilled water per well. Cellswere incubatedfor 30 min, during which they were disrupted by repeated pipetting. Lysates were serially diluted and plated on blood agar plates supplemented with hemin and menadione, and incubated anaerobically at 37°C for 10 days. Colony-forming units of invasive organisms were then enumerated. Invasionefficiency was expressed as the percentage of the initial inoculum recovered after antibiotic treatment and Ca9-22 lysis.

The invasion assay with HAEC was performed using the same procedure as above with EG2 medium.

Polymicrobial Infection

Following demonstration of monomicrobial infections with *P.gingivalis* strains, we performed experiments to develop a model of polymicrobial periodontalinfection using *P.gingivalis* and *F. nucleatum* as members of a prototype consortium, and examined the invasion characteristics and interactions of these organisms. For polymicrobial infection, *P.gingivalis* (1 x 10^7 cells/ ml) was gently mixed with an equal volume of *F.nucleatum* (1 x 10^7 cells / ml) and the organisms were allowed to interact for 5 min.For the monomicrobial control infection,*P.gingivalis* was mixed with an equal volume of MEM or EG2 medium. As a control for polymicribial infection, *E. coli* SCS110 or DH5α was pre-incubated with *P. gingivalis*.The poly- and monomicrobial inocula were added to Ca9-22 or HAEC monolayers.

The bacterial culture growth phase, viability, counts, interaction times, suspension medium, infection dose, and infection procedures were all standardized; i.e., the same preparation and infection protocols were used for all invasion assays throughout the study.

Inhibition of Bacterial Invasion

For inhibition assays with antiserum, *P. gingivalis*or*F. nucleatum*cells were preincubated with the indicated dilution of rabbit polyclonal anti-*P.gingivalis* serum for 30 min at RT prior to use in assays.

*P. gingivalis*or*F. nucleatum*cells were also preincubated with indicatedconcentrations of D-galactose(inhibitor of *F. nucleatum* adhesion/invasion)for 15 min at RT prior to use in assays.

To dissect the biochemical pathways involved, the effect of a group of metabolic inhibitors on invasion was investigated. The following inhibitors, in the solvent and at the final concentration indicated, were used. Cytochalasin D, 1 µg/ml in dimethyl sulfoxide (DMSO); nocodazole, 10 µg/ml in DMSO; staurosporine, 0.5 µM in DMSO; cycloheximide,

100 µg/ml in ethanol. The inhibitors were preincubated with the host cells for 60 min prior to addition of the bacteria and remained present throughout the invasion assay. All potential inhibitors were tested at the concentration used for possible adverse effects on the host cells, through comparison to cells without inhibitors, by examining the morphology of the cells and the confluency of the monolayer.

Preparation of F. Nucleatum Culture Filtrate

F. nucleatum was grown in BHI mediumsupplemented with 0.5 % of yeast extract, hemin (5 µg/ml) and menadione (0.5 µg/ml) to an optical density of 1.0 at 660 nm, and fluid cultures were centrifugedat 600 x *g* for 10 min. The supernate was sterilized by 0.22 µm pore size membrane (Millipore, Bedford, MA, USA)filtrationfor the preparation of bacteria-free culture filtrates. A heat-treated culture filtrate was prepared with an autoclave at121 °C for 20 min. An aliquot of the culture filtrate was mixed with a *P. gingivalis* cell suspension and incubated for 5 min at RT. The mixture was then used to infect Ca9-22 monolayers.

Confocal Scanning Laser Microscopy

In order to confirm internalization of bacteriainto host cells, confocal scanning laser microscopy (CSLM) was performed. A dual labeling technique, based on the method described by Inagaki et al. [28], was used to discriminate intracellular from extracellular bacteria. Ca9-22 cells were grown on coverslips in six-well tissue culture plates and infected with *P. gingivalis*33277 or *F. nucleatum* TDC100 for 2 h. Cells were fixed in 4% paraformaldehyde in PBS (Wako Pure Chemical Industries, Osaka, Japan) for 10 min. After washing 3 times with PBS, any excess of reactive groupsparaformaldehyde were quenched with 50 mM NH_4Cl in PBS for 10 min at RT. After washing, cells were incubated with a rabbit polyclonal anti-*P. gingivalis* or anti-*F. nucleatum* serumdiluted 1:500 in PBS–0.5% BSA for 60 min. Following incubation, coverslips were washed three times with PBSand incubated with Alexa Fluor 488 (green fluorescent dye)-conjugated goatanti-rabbit immunoglobulin G (Molecular Probes, Eugene, OR, USA) diluted 1:500for 30 min to visualize attached bacteria. Internalized bacteria were then stained by first permeabilizing Ca9-22 cells by dipping coverslips in 0.4% Triton X-100 solution for 5 min, then staining with the rabbit anti-*P. gingivalis* or anti-*F. nucleatum*antiserum followed by Alexa Fluor 568 (red fluorescent dye)-coupled goat anti-rabbit immunoglobulin G (Molecular Probes) diluted 1:500 as described above. Actin filaments were stained with Alexa Fluor 647 (blue fluorescent dye) conjugated to phalloidin (Molecular Probes) for 30 min according to the manufacturer's recommendations to visualize the cellular cytoskeleton and confirm internalization.*P. gingivalis* could be distinguished from *F. nucleatum* on the basis of cellular morphology.

Coverslips mounted in an antifading mounting medium (VECTASHEILD, Vector Laboratories, Burlingame, CA, USA) were examined by confocal scanninglaser microscopy (CSLM) using a LSM5 DUO microscope (Carl Zeiss MicroImaging, Göttingen, Germany) with a 63 x oil immersion objective. A series of 20-25 Z-stack images was scanned in

increments using excitation wavelengths of 488, 561 and 633 nm. Images were analyzed using ZEN 2008 software (Carl Zeiss).

Z stacks of thex-y sections of CLSM were processed to render a three-dimensional (3D) image using 'Iso Surface' and 'Spot Detection' functionsof Imaris 7.0.0 (Bitplane AG; Zurich, Switzerland) software.

Data and Statistical Analyses

All experiments were performed in duplicate or triplicate for each condition and repeated at least three times. Statistical comparisons were performed using a software package (InStat 3.1, GraphPad Software, La Jolla, CA, USA).

Results

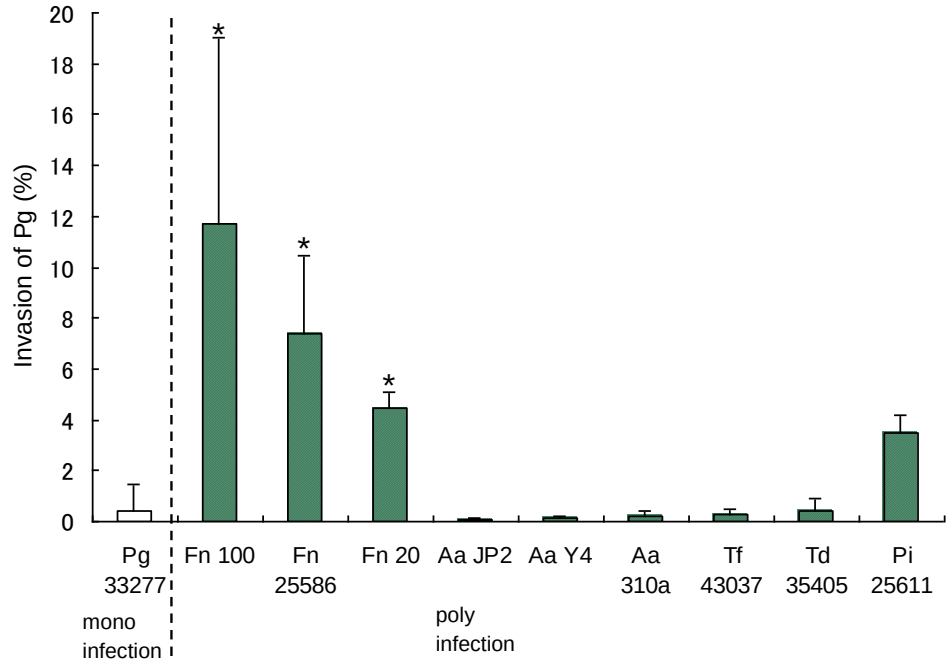
Invasion ofHost Cells by P. Gingivalis in the Poly-Infection

In polymicrobial infection experiments, several periodontopathogens were individually co-infected with *P. gingivalis* as a prototype consortium, and viable counts of *P.gingivalis*recovered from host cells after antibiotic killing were used to determine bacterial invasion. Among the pathogens, only *F. nucleatum*strains demonstrated a capacity to significantly boost*P. gingivalis* invasion of Ca9-22 cells, resulting in 3- to 8- fold increasein invasion efficiencies (Fig. 1). Co-infection with *P. intermedia* slightly enhanced invasion of *P. gingivalis*. In contrast, co-infection with *A. actinomycetemcomitans* strains or *T. forsythia* significantly abrogated invasion of *P. gingivalis*. Co-infection with *T. denticola* showed no effect on *P. gingivalis* invasion. Likewise, control *E. coli* strains had no effect on *P. gingivalis* invasion.

Similarly, co-incubation of *P.gingivalis* 33277 with *F. nucleatum* significantly boosted *P. gingivalis* invasion of HAEC. In the presence of *F. nucleatum*, *P. gingivalis* W83 which showed low invasive ability in monomicrobial infection, demonstrated dramatic increase in invasion (data not shown). Invasion of HAEC with *P. gingivalis* W83 was significantly more enhanced in the presence of *F. nucleatum* TDC100 (invasion efficiency: 8 %) than *F. nucleatum* 25586 (2%) ($P< 0.01$).

Effect of F. Nucleatum on Invasion of Other Periodontopathogens

Following demonstration of fusobacterial enhancement of *P. gingivalis* invasion, we tested whether *F. nucleatum* could also promote invasion of Ca9-22 cells by other periodontopathogens. Co-infection with *F. nucleatum* TDC100 exerted no significant effect on invasion by *A. actinomycetemcomitans*, but attenuated that by *P. intermedia*.



Values given as means ± standard deviations of triplicate independent determinations from a typical experiment.

*Statistically significantly different from monomicrobial infection ($P < 0.01$) by analysis of variance (ANOVA) with Bonferroni post test

Pg: P. gingivalis, Fn: F. nucleatum, Aa: A. actinomycetemcomitans, Tf: T. forsythia, Td: T. denticola, Pi: Prevotella intermedia

Figure 1.Invasion of human gingival epithelial cells by *P. gingivalis* 33277 in mono- or polymicrobial infectionCa9-22 cells (10^5cells) were infected with 10^7 bacteria (MOI=100).

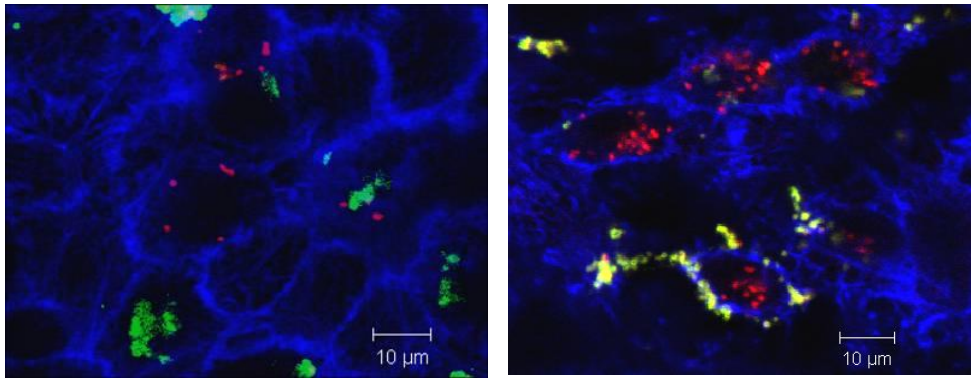


Fig 2a **Fig 2b**

Figure 2. Attachment and invasion of *P. gingivalis*into gingival epithelial cells visualizedby confocal scanning laser microscopy(CSLM) following dual labeling. *P. gingivalis* 33277 invading Ca9-22 cells were stained red, while extracellular bacteria were detected as green-yellow, with anti-*P. gingivalis* antibody. The hostcell cytoskeleton stained with phalloidin appearedblue. (a) Mono-infection by *P. gingivalis*; (b) Co-infection with *P. gingivalis* and *F. nucleatum*. Internalized *P. gingivalis* cells were stained red.

Assessment of internalization of P. gingivalis or F. nucleatum in host cells by confocal scanning laser microscopy (CSLM)

Internalization of *P. gingivalis* was initially assessed by CSLM following double staining using anti-*P. gingivalis* antibody. Thefrequency of *P. gingivalis* 33277 invadingCa9-22 cells increased in the presence of *F. nucleatum*TDC100 (Fig 2a, b).These CSLM data correlated well with the results of the antibiotic protection assays.

When infected alone, *F. nucleatum* TDC100 demonstrated ability to invade the host cells, which was significantly less than that of *P. gingivalis* 33277. Co-infection with *P. gingivalis* did not significantly alter the frequency of *F. nucleatum* invasion. Next, anti-*P. gingivalis* and anti-*F. nucleatum* antisera were used together to examine whether the ability of *P. gingivalis* to invade Ca9-22 cells was dependent upon the concomitant internalization of *F. nucleatum*. It was often observed that Ca9-22 cells contained both *P. gingivalis* and *F. nucleatum* (Fig 3). However, some of the host cells solely contained *P. gingivalis*.

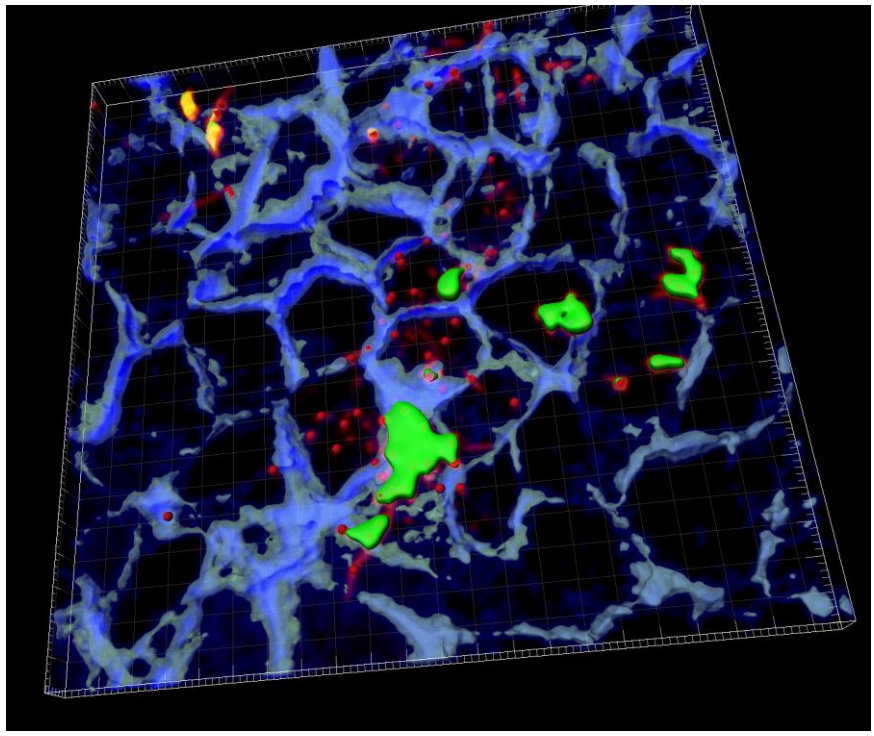


Figure 3. Attachment and invasion of *P. gingivalis*or *F. nucleatum*into gingival epithelial cells. Z stacks of the x-y sections of CLSM were processed to render a 3D image using 'Iso Surface' and 'Spot Detection'functions of Imaris 7.0.0 software. Internalized *P. gingivalis* (shown as sphere) and *F. nucleatum* (shown as longer, fusiform shape) were stained red, while extracellular bacteria were shown green-yellow. Anti-*P. gingivalis* and anti-*F. nucleatum* antisera were used. The hostcell cytoskeleton stained with phalloidin appearedblue.

Effect of F. Nucleatum Culture Filtrate or Viabilityon P. Gingivalis Invasion

In order to further analyze the fusobacterial enhancement of *P. gingivalis* invasion, we tested the effect of *F. nucleatum* culture filtrates on the host cellinvasion. Unlike co-

incubation with *F. nucleatum* viable cell suspension, the filtrates exerted no significant effect on *P. gingivalis* invasion.

To determine the effect of fusobacterial viability on *P. gingivalis* invasion, heat-killed or methanol-fixed *F. nucleatum*cells were mixed with *P. gingivalis* and co-infected Ca9-22 cells. Killing of the bacteria was confirmed by culture on blood agar plates. Co-infection with heat-killed or methanol-fixed *F. nucleatum*resulted in an increase in *P. gingivalis* invasion, although the extent was lower than co-infection with viable *F. nucleatum* cells.

Inhibition of P. Gingivalis Invasion

P.gingivalis invasion of HAEC was inhibited by anti-*P.gingivalis* serum (diluted 1:100) by approximately 70 %. The inhibition was significant, but relatively low when co-incubated with *F. nucleatum*. A similar trend was observed with Ca9-22 cells.

To determine whether the enhanced invasion of *P. gingivalis* in polymicrobial infection involves alectin-like adhesin(s), sugar inhibition assay was performed. Incubation with D-galactose reduced*P. gingivalis*invasionby approximately 40 %in polymicrobial infection experiments.

Various metabolic inhibitors previously reported to reduce *P. gingivalis* or *F. nucleatum* invasion were assessed for the ability to inhibit *Fusobacterium*-enhanced *P. gingivalis* invasion. In mono- and polymicrobial infection experiments, invasion of the host cells by *P. gingivalis* required multiple components of the host including actin, microtubule, and protein kinases (Table 1). One notable difference in inhibition profiles was observed between mono- and polymicrobial infections. Cycloheximide (which target host cell protein synthesis) significantly reduced invasion by *P. gingivalis* in polymicrobial infection experiments. This inhibitor has previously been shown to inhibit *F. nucleatum* invasion but not *P. gingivalis* invasion.

Table 1. Effects of metabolic inhibitors on *P. gingivalis* invasion of HAEC by mono- or polymicrobial infection (Saito et al, 2008)

Inhibitor	Target	Invasion of HAEC (% of untreated control) [a]	
		Mono-infection (Pg) [b]	Poly-infection (Pg + Fn) [c]
Cytochalasin D	Actin	11.5 ± 3.5*	23.0 ± 11.1*
Nocodazole	Microtubule	14.8 ± 5.0*	18.2 ± 16.4*
Saturosporine	Protein kinase	54.4 ± 5.5*	22.7 ± 17.9*, †
Cycloheximide	Protein synthesis	91.7 ± 5.5	25.7 ±8.3*, †

[a] Invasion of *P.gingivalis* 33277 relative to the level obtained in the absence of inhibitor (medium control). Values given as means ± standard deviations of triplicate independent determinations from a typical experiment.

[b] Cells were infected by *P. gingivalis* 33277

[c] Cells were infected by *P. gingivalis* 33277 and *F. nucleatum* TDC100

*Statistically significantly different from control ($P < 0.01$) byANOVA with Bonferroni multiple comparisons test

†Statistically significantly different from monomicrobial infection ($P < 0.01$) by Mann-Whitney U test

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Discussion

Emerging evidence has indicated the significance of polymicrobial infections in which selected microorganisms interact in a synergistic or antagonistic manner, impacting on pathogenesis [21, 29, 30]. We examined the ability of *P. gingivalis,* a major periodontopahogen, to invade host cells as the primary outcome in addressing the potential virulence synergism or antagonism of oral microbial consortia. Among the periodontopathogens tested, *F. nucleatum*and *P. intermedia*demonstrated ability to enhance *P. gingivalis* invasion, with varied efficiency. *F. nucleatum* was the only pathogen that showed a statistically significant enhancement, and this remarkable effect was found to be shared among *F. nucleatum* strains tested. This organismis able to adhere to and invade human epithelial cells[31]. It has been shown to enhance host cell invasion by other bacteria such as *Streptococcus cristatus* [32] or*Pseudomonas aeruginosa* [33].This prompted us to test whether *F. nucleatum* can alter invasion of host cells by other periodontopathogens. In our experimental condition, co-infection with *F. nucleatum* did not modulate invasion of Ca9-22 cells by *A. actinomycetemcomitans* or *P. intermedia*. Collectively, these results may indicate that enhancement of host cell invasion by periodontopathogensrequires some specific combinations in polymicrobial infection.

We also explored the abilities of different *P. gingivalis* strains to invade gingival epithelial and endothelial cells. *P.gingivalis* W83 has been shown to be highly virulent in experimental animal models[34, 35]. In our experimental set-up, invasive ability of *P. gingivalis* W83 into host cells was relatively low when compared to *P.gingivalis* 33277, which has been shown to be highly fimbriated but less virulent. Furthermore, *P. gingivalis* W83 displayed an invasive ability that differed in the tested cell types. Fimbriae areconsidered important in adherence and invasion by *P.gingivalis*[36]. However, it has been shown that the presence and expression of *fimA*is not sufficient for *P. gingivalis* invasion of endothelial and epithelial cells [37, 38]. The differential invasion efficiency observed for different cell types is likely due to different interactions between *P. gingivalis* and the types of cell surface receptors present on the different cell types that are involved in the invasion process.

Synergistic interactions in virulence between *F.nucleatum* and *P.gingivalis* have been observed *in vitro* and in animal models [39, 40]. In a murine orofacial infection model, heat-stable substance(s) of *F. nucleatum* has been shown to contribute to synergistic virulence with other bacteria [41]. Given these interactions, some hypothesis can be proposed regarding the mechanism(s) by which *F. nucleatum* modulates *P. gingivalis* invasion. One hypothesis is that *F. nucleatum* release extracellular substance(s) that facilitates*P. gingivalis* invasion. In polymicrobial infection of intestinal bacteria, *Campylocacter*-conditioned cell culture medium induced non-invasive *E. coli* strain to enter into enterocytes [42]. Our observation that sterile filtrates from cell cultures of *F. nucleatum* were not capable of enhancing *P. gingivalis* invasionstrongly suggests that the host cell invasion-facilitating effect was not caused by an extracellular filterable substance.Direct contact between the co-infectants and/or the host cells would be another explanation, since co-incubation with methanol-fixed or heat-killed *F. nucleatum* appeared to partially enhance the *P. gingivalis* invasion. It is possible that fusobacterial viability is not required for this enhancement.

In polymicrobial infection experiments, *F.nucleatum* TDC 100 enhanced *P . gingivalis* invasion of host cells significantly more than *F. nucleatum* type strain. A previous study from our group has demonstrated that *F. nucleatum* TDC 100 has a synergistic relationship with *P.gingivalis* and a strong biofilm forming ability [26]. Han *et al*. [31]reported that a spontaneous *lam* mutant *F. nucleatum*, defective in aggregation with human lymphocytes and coaggregation with *P.gingivalis*, was defective in attachmentto and invasion of human gingival epithelial cells, suggesting that same bacterial determinants are involved in aggregation properties and ability to invade host cells.*F. nucleatum* and *P. gingivalis* are strong coaggregating pairs, and the coaggregation may have the capacity to alter the expression of virulence factors in individual microorganisms [39]. Coaggregation between *P. gingivalis* and *F. nucleatum* is mediated by a galactoside moiety on the *P. gingivalis* surface and a lectin on the *F. nucleatum*, and inhibited by lactose, galactose and related monosaccharides [43, 44]. Threecomponents, a 40–42 kDa major Omp porin protein(FomA), and 39.5- and 30-kDa polypeptides, havebeen suggested as possible adhesins involved in thelectin-like interbacterial co-aggregation of *F. nucleatum* [44, 45]. A high-molecular-mass component,ranging from 300 to 330 kDa, has also beensuggested as a lectinthat recognizes galactose andgalactose-containing substrates[46].We have previously observed a coaggregation reaction between *P. gingivalis* 33277 and *F. nucleatum* TDC 100, that is inhibitable by galactose (unpublished data). The synergistic interactions between *F. nucleatum* and *P.gingivalis*observed we observed could be partly explained by coaggregating effect between these organisms, since galactose moderately inhibited fusobacterium-enhanced *P. gingivalis* invasion.

In our study, co-infection with *F. nucleatum* strains markedly enhanced invasion of host cells by *P. gingivalis*W83, a strain we have tested to be minimally invasive in monomicrobial infection of host cells. Rudney *et al*. [47]showed that intracellular infections of buccal epithelial cells with periodontal pathogens were uniformly polymicrobial, and proposed several scenarios regarding invasion of host cells by a consortium of oral bacteria. Invasiveness might be limited to a subset of oral species that use it as a virulence factor. Alternatively, a wide range of oral bacteria which principally live in biofilm might be capable of invasion as a means of persisting. Since species interaction appears to be widespread in oral biofilm [48-50], another alternative could be that non-invasive species gain entrance to cells by forming consortia with invasive species. It has been reported that *F. nucleatum* transports noninvasive *S. cristatus* into human epithelial cells [32]. Thus, it was tempting to speculate that *F. nucleatum* strains directly 'shuttle'*P. gingivalis* into the host cells. In our antibiotic protection experiments, other strains of *F. nucleatum* with varied invasive efficiency could also enhance *P. gingivalis* invasion. In our CSLM observations, polymicrobial infection of Ca9-22 cells by *P.gingivalis* and *F. nucleatum* not only facilitated *P.gingivalis* invasion, but also resulted in the invasion by *F.nucleatum*, although the extent of *F.nucleatum* invasion was relatively low, when compared to that of *P.gingivalis.* These results suggested that internalization of *F. nucleatum* may not be a prerequisite for enhanced invasion by *P. gingivalis*. Although anti-*P.gingivalis* serum abrogated *P.gingivalis* invasion of host cells in a monomicrobial infection setting, the extent of inhibitionwas less in polymicrobial infection. Mechanisms other than adherence signal induced by *P. gingivalis*are likely to be involved, and that interaction(s) between *F. nucleatum* and host cells may play a significant role in fusobacterium-enhanced *P. gingivalis* invasion.

Also in the inhibition experiment, cycloheximide significantly reduced invasion by *P. gingivalis* in polymicrobial infection. As this inhibitor has previously been shown to inhibit *F. nucleatum* invasion [31] but not *P. gingivalis* invasion [16], it is conceivable that host cell infection by *F.nucleatum* may pave the way for increased invasion of *P.gingivalis*.

We cannot yet clarify whether the effect exerted by the coinfectant is directed at the host cell or the *P.gingivalis*.Invasive bacteria generally gain entry by co-opting and re-directing host cell mechanisms such as endocytosis [15, 31, 51-53]. Immunomodulating roles of *F. nucleatum* have been suggested by previous studies [31, 54]. Polymicrobial infections may actually modulate the adaptive host responses, leading to more effective evasion of protective immune responses.

Conclusion

Our results demonstrate that *F.nucleatum* facilitates *P.gingivalis* invasion ofhostcells. Given the significance of the polymicrobial nature of infection in periodontal as well as cardiovascular diseases, our data provide important insights which future research can build on. Our current effort is directed at elucidating the molecular mechanisms exploited by *P. gingivalis* in polymicrobial infection of host cells.

References

[1] Socransky SS, Haffajee AD.(1992). The bacterial etiology of destructive periodontal disease: current concepts. *J Periodontol*, 63, 322-331.

[2] Beck J, Garcia R, Heiss G, Vokonas PS, Offenbacher S. (1996). Periodontal disease and cardiovascular disease. *J Periodontol*, 67 (10 Suppl), 1123-1137.

[3] Chiu B. Multiple infections in carotid atherosclerotic plaques. (1999). *Am Heart J*, 138(5 Pt 2), S534-S536.

[4] Lalla E, Lamster IB, Hofmann MA, Bucciarelli L, Jerud AP, Tucker S, Lu Y, Papapanou PN, Schmidt AM. (2003). Oral infection with a periodontal pathogen accelerates early atherosclerosis in apolipoprotein E-null mice. *Arterioscler Thromb VascBiol*, 23, 1405-1411.

[5] Gibson III FC, Hong C, Chou H-H, Yumoto H, Chen J, Lien E, Wong J, Genco CA.(2004). Innate immune recognition of invasive bacteria accelerates atherosclerosis in apolipoprotein E–deficient mice. *Circulation*, 109, 2801-2806.

[6] Haraszthy VI, Zambon JJ, Trevisan M, Zeid M, Genco RJ. (2000). Identification of periodontal pathogens in atheromatous plaques. *J Periodontol*, 71, 1554-1560.

[7] Ishihara K, Nabuchi A, Ito R, Miyachi K, Kuramitsu HK, Okuda K. (2004). Correlation between detection rates of periodontopathic bacterial DNA in carotid coronary stenotic artery plaque and in dental plaque samples.*J Clin Microbiol*, 42, 1313-1315.

[8] Kozarov EV, Dorn BR, Shelburne CE, Dunn WA, Progulske-Fox A. (2005). Human atherosclerotic plaque contains viable invasive *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans*. *Arteriosclerosis, Thrombosis and Vascular Biology*, 25,e17-18.

[9] Kang I-C, Kuramitsu HK. (2002). Induction of monocyte chemoattractant protein-1 by *Porphyromonas gingivalis* in human endothelial cells.*FEMS Immunol Medical Microbiol*,34, 311–317.

[10] Roth GA, Moser B, Roth-Walter F, Giacona MB, Harja E, Papapanou PN, Schmidt AM,Lalla E. (2007). Infection with a periodontal pathogen increases mononuclear cell adhesion to human aortic endothelial cells. *Atherosclerosis*, 190, 271-281.

[11] Darveau RP, Arbabi S, Garcia I, Bainbridge B, Maier RV. (2002). *Porphyromonas gingivalis* lipopolysaccharide is both agonist and antagonist for p38 mitogen-activated protein kinase activation. *Infect Immun*, 70, 1867–1873.

[12] Roth GA, Ankersmit HJ, Brown VB, Papapanou PN, Schmidt AM, Lalla E. (2007). *Porphyromonas gingivalis* infection and cell death in human aortic endothelial cells. *FEMS Microbiol Lett*, 272, 106-113.

[13] Uitto V, Larjava H. (1991). Extracellular matrix molecules and their recepters: an overview with special emphasis on periodontal tissues. *Crit Rev Oral Biol Med*, 2, 323-354.

[14] Hajishengallis G. (2009). *Porphyromonas gingivalis* - host interactions: open war or intelligent guerilla tactics? *Microbes and Infection*, 11, 637-645.

[15] Lamont RJ, Chan A, Belton CM, Izutsu KT, Vasel D, Weinberg A.(1995). *Porphyromonas gingivalis* invasion of gingival epithelial cells. *Infect Immun*,63, 3878-3885.

[16] Deshpande RG, Khan MB, Genco CA. (1998) . Invasion of aortic and heart endothelial cells by *Porphyromonas gingivalis. Infect Immun*, 66, 5337-5343.

[17] Kinane DF, Riggio MP, Walker KF, MacKenzie D, Shearer B (2005). Bacteraemia following periodontal procedures. *J Clin Periodontol*,32, 708–713.

[18] Tribble GD, Lamont RJ. (2010). Bacterial invasion of epithelial cells and spreading in periodontal tissue. *Perio* 2000, 52, 68-83.

[19] Brogden KA, Guthmiller JM, Taylor CE. (2005) . Human polymicrobial infections. *Lancet*, 365, 253–255.

[20] Socransky SS, Haffajee AD, Chugini MA, Smith C, Kent Jr RL. (1998). Microbial complexes in subgingival plaque. *J Clin Periodontol*, 25, 134-144.

[21] Kesavalu L, Sathishkumar S, Bakhavatchalu V, Mattews C, Dawson D, Steffen M, Ebersole JL. (2007). Rat model of polymicrobial infection, immunity, and alveolar bone resorption in periodontal disease. *Infect Immun*, 75, 1704-1712.

[22] Kolenbrander PE. (2000). Oral microbial communitites: biofilms, interactions, and genetic systems. *Annu Rev Microbiol*, 54, 413-437.

[23] [23] Bukholm G & Kapperud G. (1987). Expression of *Campylobacter jejuni* invasiveness in cell cultures coinfected with other bacteria. *Infect Immun*, 55, 2816-2821.

[24] Saito A, Inagaki S, Kimizuka R, Okuda K, Hosaka Y, Nakagawa T, Ishihara K. (2008). *Fusobacterium nucleatum* enhances invasion of human gingival epithelial and aortic endothelial cells by *Porphyromonas gingivalis*. *FEMS Immunol Med Microbiol* 54, 349-355.

[25] Saito A, Inagaki S, Ishihara K. (2009). Differential ability of periodontopathic bacteria to modulate invasion of human gingival epithelial cells by *Porphyromonas gingivalis. Microb Pathog* 47, 329-333.

[26]Saito Y, Fujii R, Nakagawa K-I, Kuramitsu HK, Okuda K, Ishihara K. (2008). Stimulation of *Fusobacterium nucleatum* biofilm formation by *Porphyromonas gingivalis*. *Oral Microbiol Immunol*, 23,1-6.

[27]Ohta K, Makinen KK, Loesche WJ. (1986) Purification and characterization of an enzyme from *Treponema denticola* capable of hydrolyzing synthetic trypsin substrates. *Infect Immun* 53, 213-220.

[28]Inagaki S, Onishi S, Kuramitsu HK, Sharma A. (2006). *Porphyromonas gingivalis* vesicles enhance attachment, and the leucine-rich repeat BspA protein is required for invasion of epithelial cells by "*Tannerella forsythia*". *Infect Immun*,74, 5023-5028.

[29]Chen PB, Davern LB, Katz J, Eldridge JH, Michalek SM. (1996). Host responses induced by co-infection with *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* in a murine model.*Oral Microbiol Immunol*, 11, 274-281.

[30]Bakaletz LO. (2004). Developing animal models for polymicrobial diseases. *Nat Rev Microbiol*, 2, 552-568.

[31]Han YW, Shi W, Huang GT, Kinder Haake S, Park NH, Kuramitsu H, Genco RJ. (2000). Interactions between periodontal bacteria and human oral epithelial cells: *Fusobacterium nucleatum* adheres to and invades epithelial cells. *Infect Immun*, 68, 3140–3146.

[32]Edwards AM, Grossman TJ, Rudney JD.(2006). *Fusobacterium nucleatum* transports noninvasive *Streptococcus cristatus* into human epithelial cells. *Infect Immun*, 74, 654-662.

[33]Pan Y, Teng D, Burke AC, Haase EM, Scannapieco FA. (2009). Oral bacteria modulate invasion and induction of apoptosis in HEp-2 cells by Pseudomonas aeruginosa. *Microb Pathog*, 46, 73–79.

[34]Neiders ME, Chen PB, Suido H, Reynolds HS, Zambon JJ, Shlossman M, Genco RJ. (1989). Heterogeneity of virulence among strains of *Bacteroides gingivalis*. *J Periodont Res*, 24, 192-198.

[35]Genco CA, Cutler CW, Kapczynski D, Maloney K, Arnold RR. (1991). A novel mouse model to study the virulence of and host response to *Porphyromonas (Bacteroides) gingivalis*. *Infect Immun*,59, 1255-12631.

[36]Amano A. (2010). Bacterial adhesins to hostcomponents in periodontitis. *Periodontol* 2000, 52, 12-37.

[37]Dorn BR, Burks JN, Seifert KN, Progulske-Fox A. (2000). Invasion of endothelial and epithelial cells by strains of *Porphyromonas gingivalis*. *FEMS Microbiol Lett*,187, 139-144.

[38]Umeda JE, Missailidis C, Long PL, Anzai D, Wikström M, Mayer MPA. (2006). Adhesion and invasion to epithelial cells by fimA genotypes of *Porphyromonas gingivalis*. *Oral Microbiol Immunol* 21, 415-419.

[39]Feuille F, Ebersole JL, Kesavalu L, Stepfen MJ, Holt SC. (1996). Mixed infection with *Porphyromonas gingivalis* and *Fusobacterium nucleatum* in a murine lesion model: potential synergistic effects on virulence. *Infect Immun*, 64, 2094-2100.

[40][Ebersole JL, Feuille F, Kesavalu L, Holt SC.(1997). Host modulation of tissue destruction caused by periodontopathogens: effects on a mixed microbial infection composed of *Porphyromonas gingivalis* and *Fusobacterium nucleatum*. *Microb Pathog* 23, 23–32.

[41] Kuriyama T, Nakagawa K, Kawashiri S, Yamamoto E, Nakamura S, Karasawa T. (2000). The virulence of mixed infection with *Streptococcus constellatus* and *Fusobacterium nucleatum* in a murine orofacial infection model. *Microbe Infect*, 2, 1425-1430.

[42] Kalischunk LD, Inglis GD, Buret AG. (2009). *Campylobacter jejuni* induces transcellular translocation of commensal bacteria via lipid rafts. *Gut Pathogens*, 1:2.

[43] Kolenbrander PE, Andersen RN. (1989). Inhibition of coaggregation between *Fusobacterium nucleatum* and *Porphyromonas (Bacteroides) gingivalis* by lactose and related sugars. *Infect Immun*, 57, 3204-3209.

[44] Kinder SA, Holt SC. (1993). Localization of the *Fusobacterium nucleatum* T18 adhesin activity mediating coaggregation with *Porphyromonas gingivalis* T22. *J Bacteriol* 175, 840-850.

[45] Shaniztki B, Hurwitz D, Smorodinsky N, Ganeshkumar N, Weiss EI. (1997). Identification of a *Fusobacterium nucleatum* PK1594 galactose-binding adhesin which mediates coaggregation with periopathogenic bacteria and hemagglutination. *Infect Immun*,65, 5231–5237.

[46] Murray PA, Kern DG, Winkler JR. (1988). Identification of a galactose-binding lectin on *Fusobacterium nucleatum* FN-2. *Infect Immun*, 56, 1314–1319.

[47] Rudney JD, Chen R, Sedgewick GJ. (2005). *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Tannerella forsythensis* are components of a polymicrobial intracellular flora within human buccal cells. *J Dent Res*, 84, 59–63.

[48] Cook GS, Costerton JW, Lamont RJ. (1998). Biofilm formation by *Porphyromonas gingivalis*and *Streptococcus gordonii*. *JPeriodontal Res*, 33, 323-327.

[49] Palmer RJ Jr, Kazmerzak K, Hansen MC & Kolenbrander PE. (2001). Mutualism versus independence: strategies of mixed-species oral biofilms in vitro using saliva as the sole nutrient source. *Infect Immun*, 69, 5794-5804.

[50] McNab R, Ford SK, El-Sabaeny A, Barbieri B, Cook GS, Lamont RJ. (2003). LuxS-based signaling in *Streptococcus gordonii*: autoinducer 2 controls carbohydrate metabolism and biofilm formation with *Porphyromonas gingivalis*. *J Bacteriol*, 185, 274-284.

[51] Sandros J, Madianos PN, Papapanou PN. (1996). Cellular events concurrent with *Porphyromonas gingivalis* invasion of oral epithelium*in vitro.Eur J Oral Sci*104, 363-371.

[52] Progulske-Fox A, Kozarov E, Dorn B, Dunn W Jr, Burks J, Wu Y.(1999). *Porphyromonas gingivalis* virulence factors and invasion of cells of the cardiovascular system. *J Periodontal Res*34, 393-399.

[53] Meyer DH, Rose JE, Lippmann JE & Fives-Taylor PM. (1999). Microtubules are associated with intracellular movement and spread of the periodontopathogen *Actinobacillusactinomycetemcomitans*. *Infect Immun*, 67, 6518-6525.

[54] Choi J, Borrello MA, Smith E, Cutler CW, Sojar H, Zauderer M. (2001). Prior exposure of mice to *Fusobacterium nucleatum* modulates host response to *Porphyromonas gingivalis*. *Oral Microbiol Immunol* 16, 338-344.

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Chapter XIII

HMGB1: A Novel Inflammatory Mediator in Chronic Periodontitis[#]

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Abstract

Periodontitis is a major chronic inflammatory disease that destroys periodontal tissue and eventually results in tooth loss. Although periodontitis is a local disease, its chronic status triggers systemic inflammatory diseases including severe type 2 diabetes, heart disease, cancer and atherosclerosis. Therefore, the development of new treatments for periodontitis contributes to the effective inhibition of systemic inflammatory diseases.

High Mobility Group Box-1 (HMGB1), a primarily nuclear protein, is present in many eukaryotic cells and is highly conserved between species. HMGB1 appears to have distinct functions in cellular systems. It acts as an intracellular regulator of transcription and plays a crucial role in the maintenance of DNA function. Extracellular HMGB1 released by various cell types (i.e. macrophages/monocytes, endothelial cells and pituicytes), or necrotic cells, stimulated by lipopolysaccharide (LPS) or tumor necrosis factor-α (TNF-α) acts as a proinflammatory cytokine through the multi-ligand receptor for advanced glycation end-products (RAGE) and toll-like receptors (TLRs) 2 and 4. Extracellular HMGB1 plays a critical role in the progression of chronic inflammatory diseases, such as septic shock, rheumatoid arthritis, diabetes and atherosclerotic lesions. Recent studies show that HMGB1 is continuously released from gingival epithelial cells modulated by TNF-α and expressed in epithelial tissues of patients with periodontitis. HMGB1 may be involved in the progression of periodontitis as a novel inflammatory mediator. Therefore, understanding the mechanisms underlying the functions of HMGB1 may lead to novel therapeutic approaches for chronic periodontitis and help to prevent systemic inflammatory diseases.

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This review summarizes the current knowledge on HMGB1, including its correlation with disease and preventive medicine.

Introduction

High mobility group box-1 (HMGB1), a primarily nuclear protein, is present in many eukaryotic cells [1] and is highly conserved between species. HMGB1 appears to have distinct functions in cellular systems. It acts as an intracellular regulator of transcription and plays a crucial role in the maintenance of DNA function [2]. Extracellular HMGB1 released from various cell types including macrophages/monocytes, endothelial cells and pituicytes, or necrotic cells [3–6], stimulated by lipopolysaccharide (LPS) or cytokines acts as a proinflammatory mediator through the multi-ligand receptor for advanced glycation end-products (RAGE) [7, 8] and toll-like receptors (TLRs) 2 and 4 [9, 10]. Extracellular HMGB1 plays a critical role in the progression of chronic inflammatory diseases such as septic shock, rheumatoid arthritis (RA) and atherosclerotic lesions [3, 8, 11, 12].

Periodontal disease, which includes gingivitis and periodontitis, is the most common chronic disorder of infectious origin known in humans, with a prevalence of 10–60% in adults depending on the diagnostic criteria used [13]. Most patients with periodontitis respond to bacterial infection by mobilizing their defensive cells and releasing cytokines such as interleukin (IL)-1β, tumor necrosis factor-α (TNF-α) and IL-6, which ultimately leads to the destruction of soft tissues and bone [14].

This article reviews the current knowledge on HMGB1, including its correlation with periodontal diseases and preventive medicine.

Periodontal Disease

Symptoms

Periodontitis is a major chronic inflammatory disease that destroys the periodontal tissue and eventually causes loss of teeth due to bacterial accumulation (dental plaque), which causes an inflammatory response [15]. Chronic and progressive bacterial infection leads to gingival connective tissue destruction and irreversible alveolar bone resorption [16]. Periodontal disease has many states and stages, ranging from easily treatable gingivitis to irreversible severe periodontitis and is increased by several risk factors such as systemic disease, medications (hypotensors, anti-epilepsy drugs and anti-cancer drugs), cigarette smoking, ill-fitting bridges, and trauma caused by occlusion [13, 17–20]. In addition to these variables, medical conditions that trigger host antibacterial defense mechanisms, such as neutrophil disorders and human immunodeficiency virus (HIV) infection, are likely to promote periodontal disease [21, 22].

The most prevalent form of periodontal disease is a mild form called gingivitis. Gingivitis is characterized by inflammation of the gums, redness, swelling, and frequent bleeding on probing [23]. More advanced forms of periodontitis are also prevalent. The symptoms are similar to those of gingivitis, but are more severe due to the greater accumulation of bacteria

and stronger inflammatory responses. Additionally, periodontitis is characterized by loss of gingival connective tissue attachments and alveolar bone resorption [24].

For diagnosing the extent of periodontal disease, probing depth is a good indicator of how far the disease has advanced [25]. In healthy periodontal tissue, there is no loss of epithelial attachment or pocket formation and the periodontal pocket is less than 2 mm deep. Periodontal pockets can extend between 4 and 12 mm. Clinically, patients with periodontal pockets of 4 mm or more are diagnosed with periodontitis. Patients with periodontal pockets of 6 mm or more are diagnosed with advanced, or severe, periodontitis. Due to mild nature of symptoms, such as gingival bleeding and attachment, loss many individuals neglect to treat their disease which, if left untreated, may progress to irreversible periodontitis and tooth loss.

Pathogenesis of Periodontal Disease

The presence of large numbers of oral bacteria can induce tissue destruction indirectly by activating host defense cells, which in turn, release mediators that stimulate the effectors of connective tissue destruction. The components of microbial plaques have the capacity to induce an initial infiltrate of inflammatory cells that includes lymphocytes, macrophages, and polymorphonuclear leukocytes (PMNs) [26, 27]. Microbial components, especially LPS, activate macrophages, which synthesize and secrete a variety of proinflammatory mediators including IL-1, IL-6, IL-8, TNF-α, prostaglandin E_2 (PGE_2) and hydrolytic enzymes [14]. Similarly, bacterial substances induce T lymphocytes to produce IL-1 and lymphotoxin (LT), a molecule with similar properties to TNF-α. These cytokines play a key role in periodontal tissue destruction through the induction of collagenolytic enzymes such as matrix metalloproteinases (MMPs) [28]. These latent collagenolytic enzymes are activated by reactive oxygen species in the inflammatory environment, leading to elevated levels of interstitial collagenase in inflamed gingival tissue.

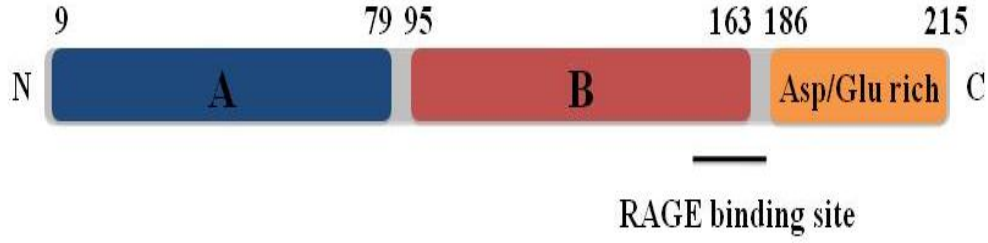
Periodontal Disease and Systemic Disease

Within the last 10 years, many studies have been published indicating a positive relationship between periodontal disease and various systemic diseases [29]. Significant associations between periodontal disease and cardiovascular disease, diabetes mellitus, preterm low birth weight and osteoporosis have been reported, bridging the once wide gap between medicine and dentistry [29]. Researchers have hypothesized the etiologic role of periodontitis in the pathogenesis of these systemic illnesses. Therefore, patients diagnosed with periodontal disease may be at higher risk due to a compromised immune system as infectious and opportunistic microbes responsible for periodontal infection may prove a burden to the rest of the body. Furthermore, these microbes can release products that elicit an inflammatory response. Periodontal lesions are recognized as continually renewing reservoirs for the systemic spread of bacterial antigens, Gram-negative bacteria, cytokines and other proinflammatory mediators. Therefore, the development of new treatments for periodontitis contributes to the effective inhibition of systemic inflammatory diseases.

HMGB1

Structure

HMGB1, also known as HMG-1 and amphoterin, is a protein encoded by the *HMGB1* gene in humans. It is a 30kDa non-histone, chromatin-binding protein ubiquitously expressed in eukaryotic cells and highly conserved across mammalian species [1]. HMGB1 comprises 215 amino acids and has a tripartite structure consisting of two DNA-binding domains (the A box and the B box) and a C-terminal tail domain containing acidic amino acids (glutamate acid and aspartic acid) [30, 31] (Fig.1A). HMGB1 binds to DNA with low affinity and can move from the nucleus to the cytoplasm depending upon the cell cycle phase [32].



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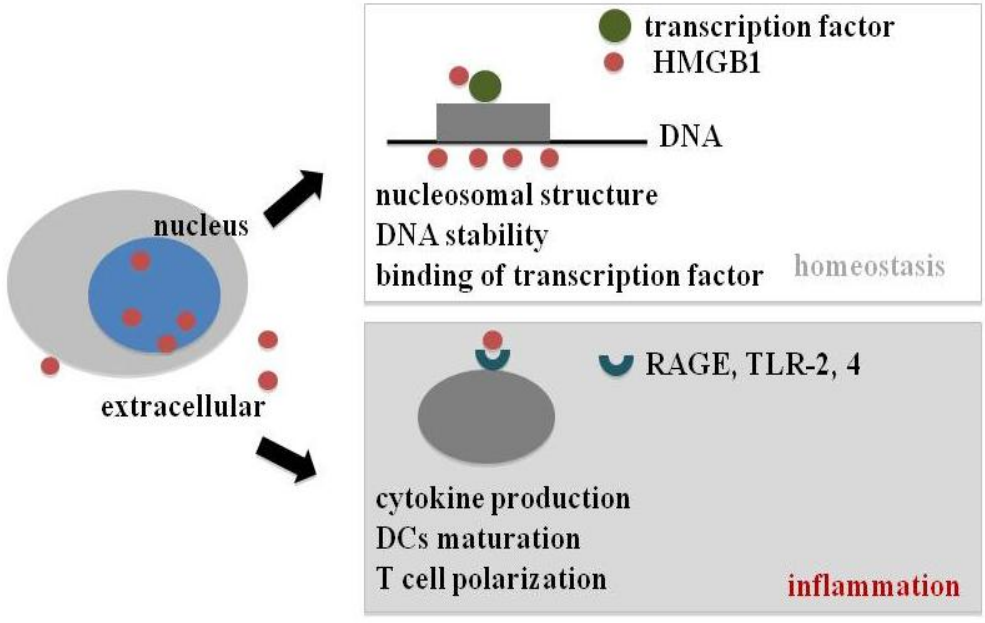

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Figure 1. Structure and functions of HMGB1. A. Structure of HMGB1, B. Functions of HMGB1

Function

HMGB1 has distinct intracellular and extracellular activities (Fig.1B). Inside the nucleus, the N-terminal lysine residue of HMGB1 is acetylated by histone acetyl transferases such as CRE-binding protein/p300 (CBP) and p300/CBP-associated factor (PCAF). HMGB1 then acts as an architectural protein that binds to DNA and regulates transcription [2]. HMGB1 recognizes particular DNA conformations rather than specific nucleic acid sequences and binds to the minor groove of the DNA helix. As a result, HMGB1 can distort DNA thereby enhancing interactions with several proteins, including NF-κB, p53, glucocorticoid receptors, progesterone receptors, and estrogen receptors [33–35]. HMGB1 appears to be essential for survival as suggested by its evolutionary conservation and the observation that HMGB1 knockout mice succumb to hypoglycemia within 24 hours of birth [36]. Thus, by recognizing DNA and modifying its structure, HMGB1 plays an important role in transcriptional regulation. Once outside the cell, HMGB1 has an entirely different role and functions as a pro-inflammatory cytokine with effects similar to those of TNF-α. The level of extracellular HMGB1 is increased in patients with severe sepsis and the administration of anti-HMGB1 antibody significantly decreases the death rate in animal models [3]. *In vitro* studies using purified HMGB1 demonstrate the immunological activities implicated in inflammatory disease. Although the activity of HMGB1 varies depending on the conditions used for its isolation and purification, both native and recombinant forms of this protein can induce the *in vitro* production of pro-inflammatory cytokines (TNF-α, IL-1, IL-6 and nitric oxide) by neutrophils, macrophages and pituicytes [5, 12, 37, 38]. This suggests that circulating HMGB1 acts as a cytokine. Despite this, however, extracellular (but not circulating) HMGB1 is regarded as a possible therapy for Duchenne muscular dystrophy. HMGB1 has the properties of “yin and yang”; it is an inflammatory cytokine with a positive therapeutic effect. Thus, HMGB1 is also referred to as “Alarmin”.

RAGE, a member of the immunoglobulin superfamily, was initially identified as a receptor for HMGB1 and is present on the surface of many cell types [39]. RAGE signaling activates the Rho and Rac small G proteins and NF-κB, which controls the expression of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6). RAGE blockade attenuates HMGB1-induced inflammatory responses [40]. Moreover, other *in vitro* studies indicate that TLRs 2 and 4, which bind to LPS, may participate in HMGB1 signaling [9, 10]. A recent study suggests that the N-terminus of thrombomodulin (TM), an anticoagulant factor, binds to HMGB1 and that TM binding to thrombin cleaves the N-terminus of HMGB1 [41], thus deactivating it.

HMGB1 and Systemic Disease

Apart from very specific situations, extracellular HMGB1 is distinctly harmful. Indeed, extracellular HMGB1 plays a critical role in the progression of chronic inflammatory diseases such as septic shock, atherosclerotic lesions, disseminated intravascular coagulation, diabetes mellitus, xenotransplantation rejection mechanisms, cerebral infarction and RA [3, 8, 41–46] (Fig.2). Therefore, agents that inhibit HMGB1 have therapeutic potential.

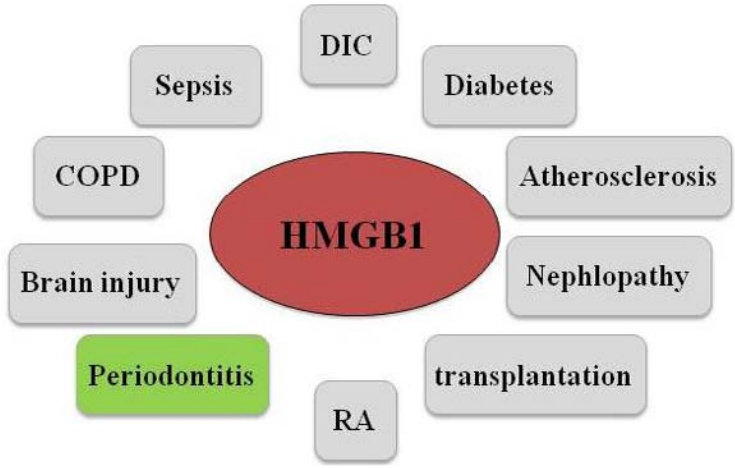

Figure 2. HMGB1 and systemic disease.

HMGB1 in Periodontitis

Periodontitis and RA share many pathological features and immunological findings. As HMGB1 plays a critical role in the progression of RA, it was speculated that HMGB1 would be involved the pathogenesis of periodontal disease.

HMGB1 and Gingival Cells

A recent study shows that gingival crevicular fluid (GCF) from periodontal patients contains HMGB1, whereas that from healthy patients does not [47]. Also, immunohistochemistry shows that HMGB1 is located in the cell nuclei of healthy tissues, but is translocated from the nucleus to the cytoplasm of gingival cells in the chronic periodontal tissues (Fig.3). This implies that HMGB1 is a highly motile protein that can shuttle to the cytosol via nuclear pores and be released from the cells into the gingival crevice during inflammation [47]. Macrophages in the connective tissue also express HMGB1 [8, 11], suggesting a potential role for the HMGB1 protein in sustaining inflammation and contributing to disease progression. HMGB1 is released from human gingival epithelial cells and from human gingival fibroblasts (HGF) stimulated with TNF-α or LPS secreted by periodontal pathogens [47, 48].

MAPK (p38MAPK, ERK1/2 and JNK) signaling pathways play important roles in inflammatory diseases such as septic shock, RA, atherosclerosis and periodontitis, as well as in other physiological processes [49]. In gingival epithelial cells, p38MAPK-mediated HMGB1 secretion is stimulated by TNF-□, consistent with a previous study showing that LPS-induced HMGB1 release is mediated through the p38MAPK signaling pathway [50]. These results suggest that continued release of HMGB1 over time following stimulation can act, at least in part, as an important amplification signal for progressive periodontal destruction.

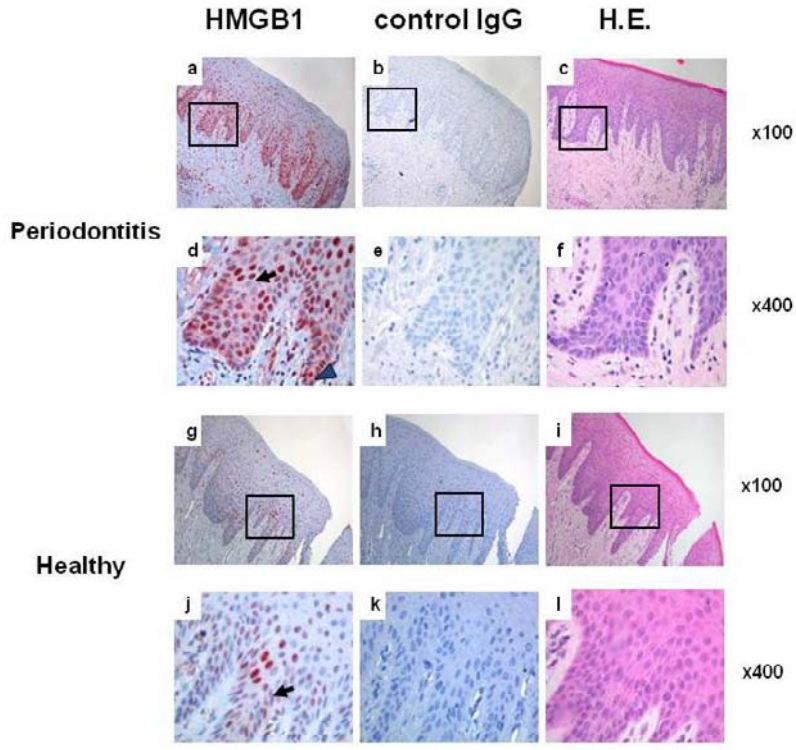

Figure 3. HMGB1 expression in inflamed gingival tissues. The arrow and arrowhead indicate HMGB1 positivity in both the nucleus and cytoplasm of gingival epithelial cells, respectively (a and d). Healthy gingival tissues show HMGB1 localization in the nucleus (arrow, g and j). Hematoxylin and eosin (H&E) staining was employed at the same time (c, f, i and l). Magnifications: x100 (upper panels); x400 (lower panels).

Additionally, HMGB1 can be released from HGF following cell death, whether apoptotic or necrotic [48]. HMGB1 is recognized as a danger signal, which can evoke an immune response in response to infection and tissue damage. Danger-signaling molecules function extracellularly by alerting the body to impending danger and initiating an appropriate host response toward tissue repair. HMGB1 can promote stem cell migration to the damaged area and cell differentiation following cardiac infarction and neural injury [51, 52]. It is also possible that HMGB1 from necrotic and apoptotic HGF may function as a danger signal in periodontal tissues by transmitting information related to bacterial infection and tissue damage in periodontitis.

Gingival cells may contribute to HMGB1 release in periodontal lesions through active release upon LPS-stimulation and diffusion out of dead cells. In periodontal disease, the invasion of connective tissues by periodontal pathogens such as *A. actinomycetemcomitans* and *P. gingivalis* is frequently observed, suggesting that high concentrations of LPS may be released into inflamed tissues by these bacteria. Gingival cells can be a source of HMGB1 during acute inflammation, when the bacterial invasion of connective tissue is severe, thus causing more cell damage. HMGB1 actively released from gingival cells in response to LPS or TNF-α, or by diffusion following apoptotic or necrotic cell death, may contribute to the pathogenesis of periodontal disease.

As reported previously, extracellular HMGB1 regulates proinflammatory cytokine production, including that of TNF-α, IL-1α, IL-1β, and IL-6, and these cytokines are highly expressed in inflamed gingival tissues [53]. Expression of RAGE, one of the receptors for HMGB1, has been detected in human gingival tissues from chronic periodontitis patients, either with or without type 2 diabetes [54], and can be induced by advanced glycation end-products (AGEs) and TNF-α [55]. Therefore, the proinflammatory effects of extracellular HMGB1 acting through RAGE may be involved in the pathogenesis of periodontitis. Alternatively, HMGB1 can bind to other molecules such as LPS and cytokines, and may enhance their activity when these complexes bind to their specific receptors [56, 57].

HMGB1 and Bone Cells

Osteoclasts, osteoblasts, and apoptotic bone cells release HMGB1 [58, 59]. HMGB1 induces inflammatory bone loss via the release of Receptor Activator for Nuclear Factor κB Ligand (RANKL), TNF-α, and IL-6. HMGB1 is a ligand for RAGE, TLR2 and TLR4, and all of these receptors are involved in the amplification of inflammation [10, 39]. Moreover, these receptors are expressed in bone, thus providing a molecular pathway for the mediation of HMGB1-induced inflammatory bone loss. HMGB1 is chemotactic for monocytes, osteoclasts and osteoblasts during endochondral bone formation. Therefore, the release of HMGB1 by bone cells may provide a molecular mechanism for the initiation and targeting bone resorption.

HMGB1 released from both gingival cells and bone cells may contribute to the pathogenesis of periodontal disease. Therefore, understanding the functional role of HMGB1 may provide a novel therapeutic approach to the treatment of periodontal disease and the prevention of systemic inflammatory diseases.

Possible New Treatments for Periodontitis

Treatment of Periodontal Disease

Once diagnosed, most periodontal diseases can be treated successfully. The therapeutic goals are to eliminate bacteria and other contributing risk factors, thereby preventing the progression of the disease and preserving the healthy state of the periodontal tissue. The recurrence of periodontitis must also be prevented. In severe cases, regeneration of the periodontal attachments must be attempted. The nonsurgical step involves a special cleaning technique called scaling and root planing. Supplemental treatment may include an antiseptic mouth rinse and other medications, either to aid the healing process, to suppress inflammation, or to further control the bacterial infection. Often, antibiotics are administered, which may offer an effective alternative to scaling and root planing. Tetracycline, or a combination of amoxicillin and metronidazole, may be used to kill a broad range of bacteria [60, 61]. However, if overused, these agents may not kill the bacteria. Another drawback to antibiotic therapy lies in the difficulty of identifying and targeting a specific pathogen due to the numerous species residing in the plaque.

Mk615, an Extract from *Ume*

Natural compounds such as catechins in green tea, naringenin, a major flavanone, in grapefruits, and polyphenols in cranberries may be useful for the prevention and treatment of inflammatory periodontal diseases [62–64]. Consequently, natural compounds with the capacity to modulate host inflammatory responses have received considerable attention, with the suggestion that they may be potential new therapeutic agents for the treatment of periodontal disease [65]. *Prunus mume Sieb. et Zucc*. is a variety of Japanese apricot known as *Ume* in Japan [66]. The health benefits of *Ume* are now being widely recognized and have been strengthened by recent studies showing that MK615, an extract from *Ume*, has strong anti-cancer and anti-proliferative effects both *in vivo* and *in vitro* [66–69]. These studies also indicate that MK615 may have strong anti-inflammatory effects.

MK615 extracted from *Ume* contains several triterpenoids, including oleanolic acid and ursolic acid, and may have anti-inflammatory effects. Recent studies have suggested that triterpenoids have both anti-tumor and anti-inflammatory effects [66–70]. Indeed, MK615 induces anti-proliferative, pro-apoptotic and pro-autophagic effects in tumor cells. Moreover, it inhibits the release of HMGB1 by LPS-stimulated RAW264.7 cells [70]. The inhibitory mechanism is mediated via the antioxidant compounds heme oxygenase-1, NQO-1 and glutathione-s transferase, which are induced by oleanolic acid. This strongly suggests that MK615 may suppress inflammation. Additionally, MK615 inhibits cytokine release, including that of TNF-α and IL-6, by *P. gingivalis* LPS-stimulated RAW264.7 cells via the inhibition of MAPK signaling, including that of ERK1/2, p38MAPK and JNK [71]. Therefore, the inhibitory mechanism appears to be mediated via MAPK signaling in *P. gingivalis* LPS-stimulated cells, and events downstream of NF-κB fail to become activated. These results support the notion that MK615 has anti-inflammatory effects.

The host inflammatory response to periodontal pathogens, notably the excessive production of cytokines, is considered to be the major factor contributing to the local tissue destruction observed in periodontitis. Consequently, therapeutic approaches that inhibit cytokine production are receiving increasing attention as options for managing chronic periodontitis. HMGB1 and pro-inflammatory cytokines such as TNF-α and IL-6 are involved in the initiation and amplification of the inflammatory process [72]. They also contribute to the pathophysiology of many inflammatory diseases, including RA, atherosclerosis and periodontitis [63]. More specifically, TNF-α plays an active role in the progression of periodontitis by inducing the expression of adhesion molecules and other mediators that facilitate and amplify inflammatory responses, stimulate MMPs, and enhance bone resorption [73]. IL-6, a multifunctional cytokine, plays an important role in regulating immune responses and bone resorption during periodontal disease. Taken together, MK615 may help to reduce the impact of the host-destructive processes mediated by cytokines such as HMGB1, TNF-α and IL-6, and may represent a useful therapeutic agent for chronic periodontitis.

Further studies are required to investigate the effects of local application of MK615 as an adjunctive treatment to conventional therapy for periodontitis patients. Such studies may lead to the development of novel periodontal therapies and improved strategies for public oral health.

Conclusion

HMGB1 is released by activated macrophages/monocytes, gingival cells, bone cells, and necrotic cells, and functions as a critical mediator of inflammatory disease. It is currently unknown whether HMGB1-mediated inflammatory responses contribute to the pathogenesis of periodontal disease. Further investigations in this area will improve our understanding of the pathophysiology of periodontitis and identify novel therapeutic strategies for the treatment of periodontitis and other inflammatory diseases. Blockade of HMGB1 activity using neutralizing antibodies or HMGB1 release using natural compounds such as cathecin and *Ume* extract (MK615) may provide an additional therapeutic target for the treatment of periodontal disease.

References

[1] Mosevitsky MI., Novitskaya VA., Iogannsen MG., Zabezhinsky MA., (1989). Tissue specificity of nucleo-cytoplasmic distribution of HMG1 and HMG2 proteins and their probable functions. *Eur J Biochem.*, 185, 303-310.

[2] Lu J., Kobayashi R., Brill SJ., (1996). Characterization of a high mobility group 1/2 homolog in yeast. *J Biol Chem*., 271, 33678-33685.

[3] Wang H., Bloom O., Zhang M., et al., (1999). HMG-1 as a late mediator of endotoxin lethality in mice. *Science*., 285, 248-251.

[4] Mullins GE., Sunden-Cullberg J., Johansson AS., et al., (2004). Activation of human umbilical vein endothelial cells leads to relocation and release of high-mobility group box chromosomal protein 1. *Scand J Immunol*., 60, 566-573.

[5] Wang H., Vishnubhakat JM., Bloom O., et al., (1999). Proinflammatory cytokines (tumor necrosis factor and interleukin 1) stimulate release of high mobility group protein-1 by pituicytes. *Surgery*., 126, 389-392.

[6] Scaffidi P., Misteli T., Bianchi ME., (2002). Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature*., 418, 191-195.

[7] Taguchi A., Blood DC., del Toro G., et al., (2000). Blockade of RAGE-amphoterin signalling suppresses tumour growth and metastases. *Nature*., 405, 354-360.

[8] Taniguchi N., Kawahara K., Yone K., et al., (2003). High mobility group box chromosomal protein 1 plays a role in the pathogenesis of rheumatoid arthritis as a novel cytokine. *Arthritis Rheum*., 48,971-981.

[9] Park JS., Svetkauskaite D., He Q., et al., (2004). Involvement of toll-like receptors 2 and 4 in cellular activation by high mobility group box 1 protein. J Biol Chem., 279, 7370-7377.

[10] Park JS., Gamboni-Robertson F., He Q., Svetkauskaite D., Kim Jy., Strassheim D., et al., (2006). High mobility group box 1 protein interacts with multiple Toll-like receptors. *Am J Physiol*., 290, C917–924.

[11] Kalinina N., Agrotis A., Antropova Y., et al., (2004). Increased expression of the DNA-binding cytokine HMGB1 in human atherosclerotic lesions: role of activated macrophages and cytokines. *Arterioscler Thromb Vasc Biol*., 24, 2320-2325.

[12] Dumitriu IE., Baruah P., Manfredi AA., Bianchi ME., Rovere-Querini P., (2005). HMGB1: guiding immunity from within. *Trends Immunol*., 26, 381-387.

[13] Papapanou P.N., (1996). Periodontal diseases: Epidemiology. *Ann. Periodontal.*, 1 (1), 1-36.

[14] Birkedal-Hansen H., (1993). Role of cytokines and inflammatory mediators in tissue destruction. J. Periodont. Res., 28, 500–510.

[15] Loe H., TheiladeE., Jensen S.B. (1965). Experimental gingivitis in man. J. Periodontol., 36, 177-187.

[16] Ranny R. (1993). Classification of periodontal disease. Periodontol 2000., 2, 13-25.

[17] Slavin J.,Taylor J., (1987). Cyclosporine, nifedipine and gingival hyperplasia. *Lancet*., 2, 739.

[18] Grossi S.G., Zambon J., Machtei E.E., Schifferle R., Andreana S., Genco R.J., Cummins D., Harrap G., (1997). Effects of smoking and smoking cessation on healing after mechanical periodontal therapy. *J. Am. Dent. Assoc*., 128 (5), 599-607.

[19] Bjorn AL., Bjorn H., Grcovic B., (1969). Marginal fit of restorations and its relation to periodontal bone level. *Odont Revy*., 20, 311-321.

[20] Glickman I., (1965). Clinical significance of trauma from occlusion. *J. Am. Dent. Assoc*., 70, 607-618.

[21] Clark R.A., Page R.C., Wilde G., (1977). Defective neutrophil chemotaxis in juvenile periodontitis. *Infect. Immun*., 18 (3), 694-700

[22] Mealey BL., (1996). Periodontal implications: medically compromised patients. *Ann Periodontol*., 1, 256-321.

[23] Greenstein G., (1984). The role of bleeding upon probing in the diagnosis of periodontal disease. A literature review. *J. Periodontol*., 55 (12), 684-688.

[24] Graves DT., Jiang Y., Genco C., (2000). Periodontal disease: bacterial virulence factors, host response and impact on systemic health. *Curr Opin Infect Dis*., 13, 227-232.

[25] Greenstein G., (1997). Contemporary interpretation of probing depth assessments: diagnostic and therapeutic implications. A literature review. *J. Periodontol*., 68 (12), 1194-1205.

[26] Kowashi Y., Jaccardand F., Cimasoni G., (1980). Sulcular polymorphonuclear leucocytes and gingival exudate during experimental gingivitis in man. *J. Periodont. Res*., 15, 151–158.

[27] Zappa U., Reinking-Zappa M., Grafand H., Espel M., (1991). Cell populations and episodic periodontal attachment loss in humans. *J. Clin. Periodontol*., 18, 508–515.

[28] Sorsa T., Ingman T., Suomalainen K., Haapasalo M., Konttinen YT., Lindy O., Saari H., Uitto VJ., (1992). Identification of proteases from periodontopathogenic bacteria as activators of latent human neutrophil and fibroblast-type interstitial collagenases. *Infect Immun.*, 60, 4491–4495.

[29] Jemin Kim., Salomon Amar., (2006). Periodontal disease and systemic conditions: a bidirectional relationship. *Odontology*, 94, 10-21.

[30] Wen L., Huang JK., Johnson BH., Reeck GR., (1989). A human placental cDNA clone that encodes nonhistone chromosomal protein HMG-1. *Nucleic Acids Res.*, 17, 1197–1214.

[31] Bianchi ME., Falciola L., Ferrari S., Lilley DM., (1992). The DNA binding site of HMG1 protein is composed of two similar segments (HMG boxes), both of which have counterparts in other eukaryotic regulatory proteins. *EMGO J.*, 11, 1055–1063.

[32] Falciola L., Spada F., Calogero S., Langst G., Voit R., (1997). Grummt I, et al. High mobility group 1 protein is not stably associated with the chromosomes of somatic cells. *J Cell Biol.*, 137, 19–26.

[33] Jayaraman L., Moorthy NC., Murthy KG., Manley JL., Bustin M., Prives C., (1998). High mobility group protein-1 (HMG-1) is a unique activator of p53. *Genes Dev.*, 12, 462–72.

[34] Agresti A., Lupo R., Bianchi ME., Muller S., (2003). HMGB1 interacts differently with members of the Rel family of transcription factors. *Biochem Biophys Res Commun.*, 302, 421–426.

[35] Boonyaratanakornkit V., Melvin V., Prendergast P., Altmann M., Ronfani R., Bianchi ME., et al., (1998). High-mobility group chromatin proteins 1 and 2 functionally interact with steroid hormone receptors to enhance their DNA binding in vitro and transcriptional activity in mammalian cells. *Mol Cell Biol*., 18, 4471–4487.

[36] Calogero S., Grassi F., Aguzzi A., Voigtländer T., Ferrier P., Ferrari S., et al., (1999). The lack of chromosomal protein Hmg1 does not disrupt cell growth but causes lethal hypoglycaemia in newborn mice. *Nat Genet*., 22, 276–280.

[37] Andersson U., Wang H., Palmblad K., Aveberger AC., Bloom O., Erlandsson-Harris H., et al., (2000). High mobility group 1 protein (HMG-1) stimulates proinflammatory cytokine synthesis in human monocytes. *J Exp Med.*, 192, 565–570.

[38] Zimmermann K., Volkel D., Pable S., Lindner T., Kramberger F., Bahrami S., et al., (2004). Native versus recombinant high-mobility group B1 proteins: functional activity in vitro. *Inflammation*., 28, 221–229.

[39] Bierhaus A., Humpert PM., Morcos M., Wendt T., Chavakis T., Arnold B., et al., (2004). Understanding RAGE, the receptor for advanced glycation end products. *J Mol Med.*, 83, 876–886.

[40] Kokkola R., Andersson A., Mullins G., Ostberg T., Treutiger CJ., Arnold B., et al., (2005). RAGE is the major receptor for the proinflammatory activity of HMGB1 in rodent macrophages. *Scand J Immunol*., 61, 1–9.

[41] Ito T, Kawahara K, Okamoto K, Yamada S, Yasuda M, Imaizumi H, Nawa Y, Meng X, Shrestha B, Hashiguchi T, Maruyama I. (2008). Proteolytic cleavage *Arterioscler Thromb Vasc Biol*., 28 (10),1825-1830.

[42] Kawahara K., Kamal KB., Unoshima M., Ito T., Kiyoshi K., Morimoto Y., et al., (2008). CRP induces high mobility group box-1 protein release through a p38MAPK in the macrophage cell line RAW264.7 cells. *Cardiovasc Pathol*.,17(3), 129-138.

[43] Kawahara K., Setoyama K., Kikuchi K., Biswas KK., Kamimura R., Iwata M., et al., (2007). HMGB1 release in co-cultures of porcine endothelial and human T cells. *Xenotransplantation*., 14, 636-41.

[44] Porto A., Palumbo R., Pieroni M., Aprigliano G., Chiesa R., Sanvito F., et al., (2006). Smooth muscle cells in human atherosclerotic plaques secrete and proliferate in response to high mobility group box 1 protein. *FASEB J.*, 20, 2555-2566.

[45] Inoue K., Kawahara K., Biswas KK., Ando K., Mitsudo K., Nobuyoshi M., et al., (2007). HMGB1 expression by activated vascular smooth muscle cells in advanced human atherosclerosis plaques. *Cardiovasc Pathol*., 16, 136-43.

[46] Kikuchi K., Kawahara KI., Tancharoen S., Matsuda F., Morimoto Y., Ito T., Maruyama I., et al., (2009). The free-radical scavenger edaravone rescues rats from cerebral infarction*The Journal of pharmacology and experimental therapeutics*., 329, 865-874.

[47] Morimoto Y., Kawahara KI., Tanchareon S., Kikuchi K., Matsuyama T., Hashiguchi T., Izumi Y., Maruyama I., (2008). Tumor necrosis factor-α Stimulates Gingival Epithelial Cells to Release High-Mobility-Group Box 1. *Journal of Periodontal Research*., 43, 76-83.

[48] Feghali K., Iwasaki K., Tanaka K., Komaki M., Machigashira M., Ishikawa I., Izumi Y., (2009). Human gingival fibroblasts release high-mobility group box-1 protein through active and passive pathways. *Oral Microbiology Immunology*., 24, 292-298.

[49] Nishikawa M., Myoui A., Tomita T., et al., (2003). Prevention of the onset and progression of collagen-induced arthritis in rats by the potent p38 mitogen-activated protein kinase inhibitor FR167653. *Arthritis Rheum*., 48, 2670-2681.

[50] Chen G., Li J., Ochani M., et al., (2004). Bacterial endotoxin stimulates macrophages to release HMGB1 partly through CD14- and TNF-dependent mechanisms. *J Leukoc Biol*., 76, 994-1001.

[51] Germani A., Limana F., Capogrossi MC., (2007). Pivotal advances: high-mobility group box 1 protein – a cytokine with a role in cardiac repair. *J Leukoc Biol*., 81, 41-45.

[52] Huttunen HJ., Kuja-Panla J., Rauvala H., (2002). Receptor for advanced glycation end products (RAGE) signaling induces CREB-dependent chromogranin exspression during neuronal differentiation. *J Biol Chem*., 277, 38635-38646.

[53] Kesavalu L., Chandrasekar B., Ebersole JL., (2002). *In vivo* induction of proinflammatory cytokines in mouse tissue by *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans. Oral Microbiol Immunol*., 17, 177-180.

[54] Katz J, Bhattacharyya I, Farkhondeh-Kish F, et al. Expression of the receptor of advanced glycation end products in gingival tissues of type 2 diabetes patients with chronic periodontal disease: a study utilizing immunohistochemistry and RT-PCR. *J Clin Periodontol* 2005; 32: 40-44.

[55] Tanaka N, Yonekura H, Yamagishi S, et al. The receptor for advanced glycation end products is induced by the glycation products themselves and tumor necrosis factor-alpha through nuclear factor-kappa B, and by 17beta-estradiol through Sp-1 in human vascular endothelial cells. *J Biol Chem* 2000; 275: 25781-25790

[56] Sha Y., Zmijewski J., Xu Z., Abraham E., (2008). HMGB1 develops enhanced proinflammatory activity by binding to cytokines. *J Immunol*., 180, 2531-2537.

[57] Youn JH., Oh YJ., Kim ES., Choi JE., Shin JS., (2008). High mobility group box 1 protein binding to lipopolysaccharide facilitates transfer of lipopolysaccharide to CD14 and enhances lipopolysaccharide-mediated TNF-alpha production in human monocytes. *J Immunol.*, 180, 5067-5074.

[58] Charoonpatrapong K., Shah R., Robling AG., Alvarez M., Clapp DW., Chen S., Kopp RP., Pavalko FM., Yu J., Bidwell JP., (2006). HMGB1 expression and release by bone *J Cell Physiol*., 207(2), 480-490.

[59] Yang J., Shah R., Robling AG., Templeton E., Yang H., Tracey KJ., Bidwell JP., (2008). HMGB1 is a bone-active cytokine. *J Cell Physiol.*, 214(3), 730-739.

[60] Hayes C., Antezak-Bouckoms A., Burdick E., (1992). Quality assessment and meta-analysis of systemic tetracycline use in chronic adult periodontitis. *J Clin Periodontol*., 9, 164–168.

[61] Van Winkelhoff AJ., Tijhof CJ., de Graaff J., (1992). Microbiological and clinical results of metronidazole plus amoxicillin therapy in *Actinobacillus actinomycetemcomitans*-associated periodontitis. *J Periodontol*., 63, 52-57.

[62] Makimura M., Hirasawa M., Kobayashi K., Indo J., Sakanaka S., Taguchi T., Otake S., (1993). Inhibitory effect of tea catechins on collagenase activity. *J. Periodontol.*, 64, 630-636.

[63] Bodet C., Grenier D., Chandad F., Ofek I., Steinberg D., Weiss EI., (2008). Potential oral health *Crit. Rev. Food. Sci. Nutr*., 48, 672-680.

[64] Bodet C., La V. D., Epifano F., Grenier D., (2008). Naringenin has anti-inflammatory properties in macrophage and *ex vivo* human whole-blood models. *J. Periodontal*. Res., 43, 400-407.

[65] Paquette DW., Williams RC., (2000). Modulation of host inflammatory mediators as a treatment strategy for periodontal diseases. *Periodontol*. 2000., 24, 239-252.

[66] Adachi M., Suzuki Y., Mizuta T., Osawa T., Adachi T., Osaka K., Suzuki K., Shiojima K., Arai Y., Masuda K., Uchiyama M., Oyamada T., Clerici M., (2007). The Japanese apricot *Prunus mume Sieb. et Zucc* (ume) is a rich natural source of novel anti-cancer substance. *Int. J. Food. Prop*., 10, 375-384.

[67] Nakagawa A., Sawada T., Okada T., Ohsawa T., Adachi M., Kubota K., (2007). New antineoplastic agent, MK615, from UME (a variety of) Japanese apricot inhibits growth of breast cancer cells in vitro. *Breast. J.*, 13, 44-49.

[68] Mori S., Sawada T., Okada T., Ohsawa T., Adachi M., Keiichi K., (2007). New anti-proliferative agent, MK615, from Japanese apricot 'Prunus mume' induces striking autophagy in colon cancer cells in vitro. *World J. Gastroenterol*., 13, 6512-6517.

[69] Okada T., Sawada T., Osawa T., Adachi M., Kubota K., (2008). MK615 inhibits pancreatic cancer cell growth by dual inhibition of Aurora A and B kinases. *World J. Gastroenterol*., 14, 1378-1382.

[70] Kawahara K., Hashiguchi T., Masuda K., Saniabadi AR., Kikuchi K., Tancharoen S., Ito T., Miura N., Morimoto Y., Biswas KK., Nawa Y., Meng X., Oyama Y., Takenouchi K., Shrestha B., Sameshima H., Shimizu T., Adachi T., Adachi M., Maruyama I., (2009). Mechanism of HMGB1 release inhibition *Int. J. Mol. Med*., 23, 615-620.

[71] Morimoto Y., Kawahara KI., Kikuchi K., Ito T., Tokuda M., MatsuyamaT., Noma S., Hashiguchi T., Torii M., Maruyama I., (2009). MK615 attenuates *Porphyromonas gingivalis* lipopolysaccharide-induced pro-inflammatory cytokine release via MAPK inactivation in murine macrophage-like RAW264.7 cell. *Biochemical and Biophysical Research Communications*., 389, 90-94.

[72] Warren JS., (1990). Interleukins and tumor necrosis factor in inflammation. *Crit. Rev. Clin. Lab. Sci.*, 28**,** 37-59.

[73] Graves DT., Cochran D., (2003) The contribution of interleukin-1 and tumor necrosis factor to periodontal tissue destruction. *J. Periodontol*., 74, 391–401.

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Chapter XIV

Risk Factors for Chronic Periodontal Diseases

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Abstract

Chronic periodontal diseases include a group of inflammatory diseases that affect periodontal supporting tissues of the teeth and encompass destructive and nondestructive conditions. Periodontal diseases are multifactorial and the role of dental biofilm in their initiation is primary. However, whether dental biofilm affects a particular subject, what form the disease takes and how it progresses, are all dependent of a wide variety of factors. Therefore, the objective of this chapter is to outline the risk factors described for the most prevalent chronic periodontal diseases (plaque induced gingivitis and chronic periodontitis) and to explain some basic concepts related to the current understanding of the role of these risk factors based on*in vitro,* animal and human studies. The review will focus on the factors that may be associated with a direct increase in the likelihood of occurrence of disease or an increase in its severity. The following factors will be discussed: 1) host characteristics, such as age, gender and race; 2) social and behavioral factors (socioeconomic status, cigarette smoking and emotional stress); 3) systemic factors, e.g. diabetes mellitus and osteoporosis; 4) genetic factors; 5) tooth-level factors (root grooves, tooth position, caries, occlusal discrepancies, iatrogenic restorations, root abnormalities and periodontal parameters); and 6) the microbial composition of dental biofilm. Finally, this chapter will also present literature-based evidence on predictive factors associated with patients and tooth susceptibility for recurrence of periodontitis

after the end of the active periodontal therapy and will examine the use of some prognostic models which may be useful for clinicians in the identification high-risk groups of patients.

Introduction

Chronic periodontal diseases include a group of inflammatory diseases that affect the periodontal supporting tissues of teeth and encompass destructive and nondestructive conditions [12]. The term chronic periodontal diseases will refer, in this chapter, to both plaque-induced gingivitis and chronic periodontitis. Plaque induced gingivitis is the inflammation of the soft tissues without apical migration of the junctional epithelium [32]. In addition, chronic periodontitis, the most frequent form of periodontitis, results in inflammation of the supporting tissues of the teeth, progressive attachment and bone lossat a slow rate, characterized by pocket formation and/or gingival recession[34]. Cross-sectional epidemiologic studies from many countries have shown that gingivitis is highly prevalent in the primary and permanent dentitions of children[7] and affects many adults[5]. Further, chronic periodontitis is also a common entity worldwide [6]. Therefore, a knowledge of the factors that may influence the transition from health to disease and of the progression of the disease through various stages of severityare important in the development of effective strategies of prevention and treatment.

Gingivitis has already been established as a consequence of dental biofilm accumulation. It is produced as the result of a general increase in the number of microorganisms and a change in the composition of the flora associated with the increasing age of the dental biofilm [99]. Several studies show that periodontitis is preceded by gingivitis and, although the accumulation and duration of microbial dental plaque biofilm will predictably lead to the development of inflammation in the nearby gingival tissues, the duration of onset and the intensity of the inflammatory process vary considerably from person to person, as well as between teeth. Albandar et al. (1998)[4] studied a periodontally high-risk group comprising 156 young subjects that were examined twice during a period of six years to study the relationship between the presence of gingival inflammation (gingival bleeding) and the occurrence of attachment loss. They found that 9.3% of sites that had gingival bleeding and 0-2 mm of attachment loss at baseline showed a longitudinal attachment loss of $\geq$ 3 mm over 6 years, whereas only 4.8% of sites with no gingival bleeding at baseline showed a corresponding attachment loss. Hence, 90.7% of sites with gingival bleeding at baseline did not show any clinical attachment loss during the study period. This study showed that not all sites with gingival inflammation developed periodontitis during the study period. Thus, predisposition to periodontitis development varies significantly and may possibly be influenced by other factors. However, defining the factors involved in initiation and progression of chronic periodontitis is a more complex issue.

Chronic periodontitis is a multi-factorial disease. While the role of bacteria is primary, a number of host-related factors have been hypothesized as influencing its diverse clinical presentation and rate of progression [72]. Loe et al. (1986)[100], in a longitudinal study, evaluated a Sri Lanka population never exposed to any programs or incidents related to the prevention and treatment of dental diseases. This population did not practice any conventional oral hygiene measures. Three subpopulations were identified: 1) individuals with rapid

progression of periodontal disease (8%); 2) individuals with moderate progression (81%); and 3) a group who exhibited no progression (11%). When another longitudinal study was made comprising a sample of middle-class Norwegian men who had the benefit of a comprehensive health care program, a group that represented an extreme condition of periodontal maintenance when compared to the Sri Lanka population, two subpopulations (moderate disease and no disease) were found, despite the severity of attachment loss [164]. These studies illustrate significant differences in the pattern and rates of attachment loss among individuals, even when they receive regular and adequate professional and personal health care. Based on the evidence above, the identification of factors involved in the initiation and progression rate of chronic periodontal diseases has been the focus of considerable research in recent times.

Chronic inflammatory periodontal diseases have several etiological factors for which a plausible biological model of effect exists. The term risk factors is commonly used and it refers to an aspect of personal behaviors or lifestyle, an environmental exposure, or an inborn or inherited characteristic, which on the basis of epidemiological evidence is known to be associated with a health-related condition [99]. The presence of a risk factor implies a direct increase in the likelihood of a disease occurring [95]. Prospective longitudinal studies, and in particular clinical trials, provide the most powerful evidence for the existence, and the amount, of risk. However, in most cases these types of studies are not easily conducted. For this reason, most evidence for the existence of possible risk factors for periodontal diseases comes from cross-sectional studies. Although the identification of risk factors for disease is unfeasible using cross-sectional studies, when a proper study design is employed, these studies can provide valuable information on the presence or absence of an association between the variables under study and the occurrence of periodontal diseases. In order to make a distinction between the results of the different types of studies, it is customary to refer to significant effects assessed in cross-sectional studies as associations, whereas effects disclosed using case-control studies and prospective studies have been referred to as risk determinants, risk indicators, or risk markers [8].

Usually, the overview of factors associated with chronic periodontal diseases is systematically presented as host characteristics, social and behavioral factors, systemic factors, genetic factors, tooth-level factors and microbial factors [126]. In addition to the investigation of these factors at the onset of chronic periodontal diseases, longitudinal studies of patients treated for periodontitis try to determine the patient's susceptibility to disease recurrence [64, 96]. As a result, the prognostic factors (disease predictors), defined as characteristics related to the progression of preexisting disease [133], have been the subject of much discussion. The identification of groups and individuals at risk for disease progression during maintenance therapy still represents one of the greatest challenges in the management of periodontal patients. Thus, prognostic models aimed at identifying high-risk individuals or teeth in a clinical setting have been described [56, 91]. A question remains about the safety of these models routinely used to help clinicians in decision-making.

Host Characteristics

Age

Several epidemiological studies have clearly demonstrated an increase in the prevalence (percentage of persons), extent (percentage of teeth per person) and severity of periodontal attachment loss with increasing age [6, 9]. Papapanou et al. (1988) [132] examined full-mouth radiographs from 531 dentate individuals aged 25-75 years and found that bone loss increased with age. Moreover, two large epidemiological studies estimated the prevalence and extent of periodontal diseases in the United States using data from the National Health and Nutrition Examination Survey (NHANES) in the years 1985 to 1986 and 1988 to 1994 [6, 23]. It was demonstrated that 48.6% of persons 35 to 44 years old and 77.3% of those 55 to 64 years old had $\geq$ 3 mm attachment loss in the first survey. The same trend was observed in the second study, in which 48.5% for the 40 to 49 year old cohort and 74.8% for the 60 to 69 year old group had $\geq$ 3 mm attachment loss. Regarding the healing of periodontal tissues following periodontal therapy, Lindhe et al. (1985) [97] evaluated 62 patients and reported that, although age did not seem to have a significant effect on the results of periodontal treatment, there was a tendency for younger patients to have a shallower probing depth and gain more periodontal attachment than older patients.

With increasing age, people have to cope with a lifelong antigenic burden encompassing several decades of evolutionary unpredicted antigenic exposure, with a major impact on survival and frailty. In fact, there is a peculiar chronic inflammatory status characterizing aging, which has been denominated by Franceschi et al. (2000)[57] as inflamm-aging, and which is considered a random process detrimental for longevity, leading to long-term tissue damage, and related to a wide range of age-related diseases, including neurodegeneration, atherosclerosis, diabetes and osteoporosis among others, which share an inflammatory pathogenesis. It may therefore be speculated that this phenomenon may also affect the periodontium, in which after a lifetime's challenge by oral periodontopathogenic bacteria and their virulence factors, periodontal tissues may develop an intense subclinical inflammatory process, but also lead to healing/regeneration outcomes after periodontal therapy [15]. In vitro studies have clearly demonstrated an age-related decrease in the proliferation of periodontal ligament cells [15, 166]. Further, aging is able to modulate the expression of genes reported to participate in periodontal homeostasis (e.g. cytokines, metalloproteinases and their inhibitors and bone-related genes) by periodontal ligament cells [14, 15]. It is important to remember the role of periodontal ligament cells on periodontal health and disease because of their ability to proliferate, migrate and synthesize several components of the periodontium and also participate in the protective host mechanism that prevents periodontitis or impedes its progression [60]. Little information on the influence of aging on the periodontium is provided by animal studies. It has been documented that the periodontal ligament presented decreased cell density and collagen synthesis, and also a decreased number of cells in the osteogenic layer of the alveolar bone has also been reported [135, 165].

Despite the well-documented loss of attachment with increasing age and the rationale behind the association, the question as to what extent aging affects periodontal homeostasis is still a controversial issue in the periodontal literature. A number of arguments have been used against the presumed association. First, there are no marked increases in the probing depth

with age. Furthermore, the prevalence of moderate and advanced periodontitis increased in patients up to approximately 65 years of age, remaining steady until they were approximately 80 years of age, and decreasing thereafter [7]. There is also an indication that the effect of age may be reduced after adjusting for the effects of other confounders [1]. And finally, a diminished ability to perform daily oral hygiene activities has been blamed for the increased prevalence of periodontitis in older individuals [139].

Despite the questions that remain to be examined before consider aging as a risk factor for periodontal diseases, it may be reasonable to suggest that age is a good indicator of the degree of periodontal tissue loss that occurs due to periodontal diseases. However, more studies are needed to clarify the role of aging as a risk factor for the development and progression of periodontal tissue loss and in tissue regeneration following therapy [10].

Gender

Epidemiological surveys show an association between gender and attachment loss in adults, with men having a higher prevalence of and more severe periodontal destruction than women. In the NHANES I survey, a better periodontal status was reported for females than males in all age groups [9]. Subsequently, Hyman & Reid (2003) [76], in a study of risk factors for periodontal attachment loss among adults in the NHANES III survey, confirmed after adjustment for confounding variables, that males were at increased risk of attachment loss, deeper probing depths and a higher prevalence of periodontitis. Attachment loss thresholds of ≥ 3 mm, ≥ 4 mm and ≥ 5 mm were noted in 23%, 44% and 55% more males than females, respectively. This is attributed to a poorer standard of oral hygiene adopted by men and it is likely that hormonal and other physiological and behavioral differences between the two genders may also contribute to the higher risk for periodontal diseases in males than females [8]. Moreover, genetic predisposing factors have been related to the increased prevalence in males [10].

Race / Ethnicity

The level of attachment loss is influenced by race / ethnicity, although the exact role of this factor is not fully understood. Certain racial / ethnic groups, particularly subjects with an African or Latin American background have a higher risk of developing periodontal tissue loss than other groups [10]. In the United States, subjects of African and Mexican descent have a greater attachment loss than Caucasians [6]. However, the increased risk of periodontitis in certain racial/ethnic groups may be partly attributed to socioeconomic, behavioral and other disparities [143]. Moreover, there is evidence that increased risk may also be related to biologic/genetic predisposition [10]. A number of studies evaluating confounding variables have failed to find any differences in periodontitis prevalence and severity between different ethnic/racial groups [37, 76, 106, 107]. For example, Craig et al. (2001) [37] evaluated periodontitis progression rates among three ethnic / racial groups, Asian, African and Hispanic Americans, over a 2-month period. No significant differences in rate of attachment loss were observed between the three groups.

Social and Behavioral Factors

Socioeconomic Status

Socioeconomic status is an important risk indicator of periodontal disease. Individuals with a low socioeconomic status have a higher occurrence of attachment loss and probing depth than those with a high socioeconomic status. Drury et al. (1999) [46] used an index comprising the individual's education attainment and family economic status and divided the United States population into four socioeconomic groups. They found that the prevalence of gingivitis and loss of attachment of ≥ 4 mm increased with the decrease in socioeconomic level. Furthermore, Dolan et al. (1997) [44] measured the attachment loss in 761 adult subjects and related these measurements to socioeconomic status and other potential risk indicators. They found that low income and residing in a rural area were significant risk indicators for attachment loss. Thus, it may be suggested that measurements of socioeconomic status, including income, education levels and urban status are fairly good risk indicators for periodontal diseases. Groups with a low socioeconomic status are at higher risk of having periodontal diseases than groups with a high socioeconomic status, and the higher level of risk in this group seems to be attributable to behavioral and environmental factors.

Smoking

It is now well established that tobacco use is among the most important, if not the most important, preventable risk factor in the incidence and progression of periodontal diseases. Cigarette smoking is associated with a two to eight-fold increased risk of periodontal attachment and/or bone loss, depending on the definition of disease severity and smoking dose [158]. For example, with the aim of examining the relationship between cigarette smoking and periodontitis and of estimating the proportion of periodontitis in the United States adult population that is attributable to cigarette smoking, Tomar & Asma (2000) [178] analyzed the data of 12,329 individuals from the NHANES III. In this study, current smokers were four times as likely to have periodontitis (the presence of ≥ 1 site with clinical periodontal attachment level ≥ 4 mm and probing depth ≥ 4 mm) compared to nonsmokers after adjusting for age, race or ethnicity, income, and educational level. Heavy smokers (≥31 cigarettes per day) had a greater risk than light smokers (≤9 cigarettes per day) with estimated odds ratios of 5.6 and 2.8, respectively. When a stricter definition of periodontitis was combined with heavy smoking in a Swedish population, the relative risk of disease ranged from 9.8 to 20.3 [19].

Summarizing the clinical findings in smokers, the gingival inflammatory response is dampened in smokers compared to non-smokers, as evidenced by a fibrotic appearance to the tissues and fewer sites that bleed upon probing smokers [18, 42]. Levels of supragingival calculus tend to be higher in smokers than in nonsmokers. This finding was independent of plaque levels. It is therefore possible to hypothesize that smoking may affect the mineralization rate of calculus [17]. Further, smokers have higher mean probing depths and more sites with deep probing [179, 182]. In addition, gingival recession is greater in smokers

compared to nonsmokers [25]. Smokers have two to four times more teeth with furcation involvement [117] and demonstrate a greater loss of alveolar bone height [16]. Finally, smokers with periodontitis have a greater loss of teeth than patients with periodontitis and no history of smoking [52].

Smoking is also associated with periodontal attachment loss in individuals who are usually considered at lower risk because of their relatively young age. Rosa et al. (2008) [154], in a parallel-arm prospective study with eighty-one students considered not to have periodontitis, showed a greater clinical attachment loss and a lower mean alveolar bone height in the smokers compared to nonsmokers. Further data has revealed that even passive smoking, the exposure to environmental tobacco smoke in the home and/or workplace, has recently been associated with periodontitis. Persons exposed to tobacco had a 1.6 times greater chance of having periodontal disease compared to individuals not exposed to second-hand smoke [11]. Further, tobacco use has an adverse effect on the full spectrum of periodontal treatment approaches, ranging from mechanical debridement, local and systemic antimicrobial therapy to surgery and regenerative procedures [80, 90,144]. Interestingly, the deleterious effects of tobacco smoking may be suppressed by its cessation, despite the impossibility of reversing its past effects. In the study conducted by Tomar & Asma (2000) [178], the relative risk for developing periodontal disease was reported to be 3.97 for smokers and 1.68 for former smokers. In addition, among former smokers, the risk decreased with the number of years since quitting (3.22 after 2 years and 1.15 after 11 years).

Animal studies provide a basis of support for the evidence from human studies, since they permit the control of confounders such as behavioral and systemic factors that may also influence periodontal disease progression. Nociti et al. (2000) [122] showed, using a rat model, that nicotine administration associated with plaque infection increased the rate of periodontal loss. Subsequently, the authors, aiming to answer the question as to whether nicotine concentration could be critical in promoting a dose-dependent response, evaluated the effect of daily administration of high doses of nicotine on the bone loss rate in the furcation region of rats by histometric analysis [123]. Nicotine concentrations administered in this study were intended to reproduce the highest nicotine concentrations found in commercially available cigarette brands. The data suggested that nicotine was able to heighten the rate of bone loss in a dose-dependent manner in ligated and non-ligated teeth. However, nicotine is just one of the 2000-3000 potentially toxic substances in tobacco smoke, which presents a complex mixture of substances such as acrolein, acetaldehyde, carbon monoxide and hydrogen cyanide [158]. Therefore, in order to investigate the influence of cigarette smoke as a whole, the researchers used a cigarette smoke exposure chamber, an acrylic device where the animals were forced to breathe the cigarette smoke-contaminated air [26]. In this study, the authors first demonstrated that cigarette smoke inhalation significantly increased bone loss resulting from ligature-induced periodontitis. Furthermore, data analysis demonstrated that the cessation of cigarette smoke inhalation might positively affect the rate of bone loss resulting from periodontitis [27]. The results of these preclinical studies have thus reinforced previous clinical studies, minimizing possible confounding factors that may exist in human studies.

The mechanisms by which cigarette smoking influences the initiation and progression of periodontitis are not fully understood. It seems that tobacco smoke may affect both the composition of the microflora and the host response. Regarding microflora, while several investigators have reported no significant differences in the incidence and distribution of

periodontal pathogens in the plaque biofilm of smokers [46], other studies have demonstrated significant differences in the recovery rates of periodontal pathogens in smokers [191]. Of particular interest are recent studies which demonstrate a high recovery rate of periodontal pathogens in shallow periodontal pockets and on oral mucous membrane [51, 69]. In addition, a smaller reduction in periodontal pathogens was reported in smokers than in nonsmokers, following scaling and root planning [181]. These studies points to the role of smoking in altering the load environment of the shallower pockets, thereby promoting the growth of these microbial species, as well as possible alterations in the host response that would allow for the growth of these specific microorganisms.

The influence of tobacco smoke on host response may occur in two areas: the periodontal pocket and the tissue. The first host response events occur in the periodontal pocket; it appears that cigarette smoking may tip the balance even further away from the protective functions of neutrophils and antibodies in the periodontal pockets and towards greater destructive activity [158]. For example, several studies have demonstrated reduced imunoglobulin G and imunoglobulin A levels in smokers versus nonsmokers [58, 146]. Furthermore, the effects of smoking on neutrophil function have demonstrated impaired phagocytosis, chemotaxis in neutrophils exposed to acute levels of tobacco smoke [35, 105] and increases in the release of potentially destructive oxidative products, such as superoxide and hydrogen peroxide [156]. The next stage of pathogenesis occurs when the bacterial plaque biofilm has overwhelmed the host defenses in the periodontal pocket and the bacterial products penetrate the underlying soft tissues. Here, the balance between protection and destruction is mediated largely by the type of cytokine pattern secreted by monocyte cell population. The preponderance of evidence has suggested that smoking will tip the balance toward a more inflammatory/destructive profile. For example, in vitro studies have demonstrated high secreted levels of interleukin-1β in isolated mononuclear blood cells when exposed to in vitro smoke [157]. In addition, nicotine, whether or not in association with lipopolysaccharide from periodontopathogenic bacteria, has been shown to increase interleukin-6 and interleukin-8 production by human gingival fibroblasts [185]. In vivo, César-Neto et al. (2005) [27] indicated that smoking modulation of bone destruction in periodontal disease may involve reduced levels of anti-inflammatory and anti-resorptive factors, such as interleukin-10 and osteoprotegerin, respectively, and may also involve high levels of pro-inflammatory cytokines, such as interleukin-6. However, in clinical studies, the results of the effects of tobacco smoke on inflammatory components have been inconclusive.

Emotional Stress

Stress is a state of physiological and psychological strain caused by adverse physical, mental, or emotional, internal or external stimuli that tend to disturb the functioning of an organism and which the organism naturally desires to avoid [45]. Whether or not a subject exhibits a stress response depends on a myriad of factors, including coping behaviors, genetic predisposition, concomitant stressors, levels of social support, and other lifestyle factors. Stress is compatible with good health, which is necessary to cope with the challenges of everyday life. Problems start when the stress response is inappropriate to the size of the challenge, producing neuroendocrine and biochemical changes that result in significant adverse effects on the proper functioning of the immune system [38, 151]. Potential effects of

the stress response that may be observed, or even measured, include anxiety, depression, impaired cognition and altered self-steem. Stressful stimuli can induce a set of reactions that produce effects on virtually all body systems [20]. Exposure to stress may affect the host immune response, making the individual more susceptible to the development of unhealthy conditions that damage periodontal health [137].

The most documented association between stress and periodontal disease is the one between acute forms of necrotizing gingivitis and periodontitis. An increased incidence of these conditions has been amply documented in military personnel during stressful activities and in students during examination periods [62, 67]. The association between stress and chronic periodontitis has been investigated. Wimmer et al. (2002) [188] conducted a retrospective case-control study of 89 patients with different forms of chronic periodontitis undergoing treatment. All participants completed a stress coping questionnaire, which served as a psychodiagnostic survey aimed at collecting data on stress coping strategies. The results showed that periodontitis patients with inadequate stress behavior strategies (defensive coping) are at greater risk for severe periodontitis. Later, the researchers discovered, by means of a 24-month prospective clinical trial, that passive coping strategies were more pronounced in advanced disease, as well as in cases of poor response to nonsurgical periodontal treatment. Patients with active coping modes had a milder form of the disease and a more favorable course of treatment [189]. A systematic review of case-control, cross-sectional and prospective studies examining psychologic factors, such as stress and depression and periodontal disease indicated that 57.1% of the studies reported a positive correlation between stress or other psychologic factors and periodontal disease, and that 14.2% did not [137]. In addition, a subsequent study confirmed the association between stress and depression and periodontal destruction [155]. The weight of evidence therefore seems to suggest an association between stress and periodontal health.

The biologic plausibility of such an association is not as yet completely elucidated. Stress can result in the dysregulation of the immune system, mediated primarily through the hypothalamic-pituitary-adrenal axis. Activation of hypothalamic-pituitary-adrenal axis by stress results in the release of an increased concentration of corticotrophin-releasing hormone from the hypothalamus. Corticotrophin-releasing hormone, in turn, acts on the anterior pituitary, resulting in the release of adrenocorticotrophic hormone (corticotrophin). The adrenocorticotrophic hormone then acts on the adrenal cortex and causes the production and release of glucocorticoid hormones (predominantly cortisol) into the circulation. The glucocorticoids then produce a response, modifying cytokine profiles, elevating blood glucose levels, and altering levels of certain growth factors [116]. The second major pathway to be activated is the sympathetic nervous system. Stress activates the nerve fibers of the autonomic nervous system, which innervate the tissues of the immune system. The nerve bodies secrete their products (cathecolamines) directly into the bloodstream. The release of catecholamines results in the hormonal secretion of norepinephrine and epinephrine from the adrenal medulla, which results in a range of effects that may act to modulate immune responses [116]. Eventually, the impact of stress on periodontal disease may be modulated by health-impairing behaviors that include neglecting oral hygiene practices, increased consumption of cigarettes and alcohol, disturbed sleeping patterns and bruxing [116].

Animal studies reinforce the hypothesis of a relationship between stress and periodontal disease by means of the influence of stress on the immune system via nervous and neuroendocrine because the model makes it possible to exclude the impact of various

behavioral changes, such as smoking and less effective oral hygiene. Takada et al. (2004) [171] demonstrated that stress modulated the progression of periodontal inflammation and increased alveolar bone loss. More recently, Peruzzo et al. (2008) [138] showed, on the basis of the same rat model of restraint stress, that chronic stress increased bone loss resulting from a ligature-induced periodontitis by a local increase in proinflammatory and proresorptive factors.

Based on the evidence described above, although further well-controlled prospective clinical trials are still required to definitively define stress as a risk factor for periodontitis, most studies point to the association between stress and periodontal disease. Stress management, therefore, may be a valuable component for current periodontal practice.

Systemic Factors

Diabetes Mellitus

Diabetes mellitus is a clinically and genetically heterogeneous group of metabolic disorders manifested by abnormally high levels of glucose in the blood [111]. It is a highly prevalent metabolic disorder; with 150 million cases estimated worldwide, which constitutes a global public health burden [142]. Diabetes is divided into two main forms: type 1 diabetes mellitus (formerly insulin-dependent diabetes mellitus) and type 2 diabetes mellitus (formerly non-insulin-dependent diabetes mellitus). Type 1 diabetes is caused by the immune-mediated destruction of the insulin-producing pancreaticβ cells and accounts for 10% to 15% of all cases of diabetes. The more common form, type 2 diabetes, results from a combination of impaired insulin production and insulin resistance. Both forms of the disease are associated with a range of complications that increase the morbidity and mortality of affected individuals [142].

Periodontal disease has been called the sixth complication of diabetes, a view supported by several reviews which conclude that the bulk of evidence indicates there is a direct relationship between diabetes mellitus and periodontal diseases [101]. The presence of diabetes mellitus is often associated with increased gingival inflammation. Karjalainen & Knuuttila (1996) [83] observed that poorly controlled diabetes mellitus in children had higher levels of gingival inflammation than did well-controlled patients, regardless of plaque levels. Moreover, gingival bleeding significantly decreased after two weeks of insulin treatment of newly diagnosed type 1 diabetic children and adolescents. Recently, Dakovic & Pavlovic (2008) [40] confirmed that gingival inflammation is more evident in children and adolescents with type I diabetes mellitus than in healthy ones. An increased risk of periodontitis for individuals with diabetes has also been documented in several studies. The relation between diabetes and periodontal health status was first determined in a population of Pima Indians, where subjects with type 2 diabetes have an approximately three-fold increased risk of attachment loss [53]. Moreover, in a 2-year longitudinal study of the Pima Indian population, Taylor et al. (1998) [173] found that individuals with type 2 diabetes had an increased risk of progressive alveolar bone loss compared with non-diabetic subjects. The study also showed that the level of metabolic control had a significant effect on disease progression. Increased risk for progressive attachment and bone loss in poorly controlled diabetic patients have been

confirmed in a meta-analysis of studies in various populations [134] and in more recent studies such as that conducted by Novak et al. (2008) [124].

Disease progression following periodontal treatment may also be related to metabolic control. Tervonen & Karjalainen (1997) [175] found that a group of type 1 diabetics with poor metabolic control had a significantly greater recurrence of deep probing depths 12 months after treatment than subjects with good or moderate diabetic control and non-diabetic controls. However, metabolically well-controlled diabetics responded to non-surgical and surgical periodontal therapy in a manner similar to that in which healthy controls responded [30, 186].

Many potential mechanisms have been studied by which diabetes could affect the periodontium. There are few differences in the subgingival microbiota between diabetic and nondiabetic patients with periodontitis [161, 162]. This suggests that alterations in the host immunoinflammatory response to potential pathogens may play a predominant role. Diabetes may result in impairment of neutrophil adherence, chemotaxis, and phagocytosis, which may facilitate the persistence of bacteria in the periodontal pocket and significantly increase periodontal destruction [108, 110]. While neutrophils are often hypofunctional in diabetes, these patients may have a hyper-responsive monocyte/macrophage phenotype, resulting in a significantly increased production of pro-inflammatory cytokines and mediators [159, 160]. This hyperinflammatory response results in high levels of pro-inflammatory cytokines in the gingival crevice fluid. In addition, high glucose concentrations induce non-enzymatic glycation and oxidation proteins, such as collagen and lipids, resulting in the accumulation of advanced glycation end-products (AGEs) in diabetic tissues, including periodontal tissues. The AGEs, through their receptors (RAGEs), may also induce the expression of pro-inflammatory cytokines. The elevated pro-inflammatory cytokines in the periodontal environment may play a role in the increased periodontal destruction seen in many people with diabetes [111]. For example, Duarte et al. (2007) [50] showed an overexpression of interleukin-1β and interleukin-6, potent pro-inflammatory cytokines, in gingival tissues of diabetic patients diagnosed with chronic periodontitis [111].

In conclusion, studies indicate that diabetics with poor glycaemic control have an increased risk of periodontitis and disease progression.

Osteoporosis

Osteopenia and osteoporosis are systemic skeletal diseases characterized by low bone mass and micro-architectural deterioration with a consequent increase in bone fragility and susceptibility to fracture. According to the World Health Organization, osteoporosis is considered to be present when mineral density is 2.5 standard deviation(SD) or more below the mean for normal young Caucasian women. Further, osteopenia is defined as a bone density level between 1 and 2.5 SD below normal bone density [82]. Both osteopenia and osteoporosis are grave public health concerns, particularly associated with estrogen deficiencyamong postmenopausal women. The risk factors for osteoporosis include many risk factors associated with advanced periodontal disease [61]. Since both osteoporosis and periodontal diseases are bone resorptive diseases, it has been hypothesized that osteoporosis could be a risk factor for the progression of periodontal disease.

The effects of osteoporosis induced by an estrogen-deficient state have been widely studied in a rat model. Bilateral ovariectomies of female rats were able to induce this condition. Tanaka et al. (2002) [172] histomorphometrically investigated the alveolar bone following estrogen deficiency and showed osteoporotic changes and thin alveolar bone proper in the interradicular septum of the first molar of ovariectomized rats. Later, Duarte et al. (2006)[49] confirmed that an estrogen-deficient state may negatively affect the tooth-supporting alveolar bone, resulting in a lower density of alveolar bone than that observed in estrogen-sufficient animals. It has also been shown that an estrogen-deficient state may significantly increase bone loss resulting from ligature-induced periodontitis and also at healthy sites [47, 48].

There have been a number of reports on the mechanisms involved in the estrogen regulation of bone metabolism. Since estrogen receptors in osteoblasts and osteoclasts were discovered [54, 128], it is believed that estrogen has a direct skeletal effect. It has also been shown that estrogen has an important role in controlling bone resorption through its action on osteoprotegerin (OPG) and the receptor activator of nuclear factor kB ligand (RANKL) mechanism [94, 189], as well as on bone-regulating factors such as interleukin-1, interleukin-6 and tumor necrosis factor [129, 63].

Animal studies have established a clear association between osteoporosis and oral bone density or osteoporosis and periodontitis-induced bone loss. In humans, the data gathered on the mostly cross-sectional studies appears to confirm a relationship between systemic and oral bone mineral density. For example, in a classic series of studies, Kribbs et al. (1983, 1989, 1990) [87, 88, 89] addressed this relationship in both normal and osteoporotic women. Although the technology used in those studies reflects the time at which the studies were carried out, they indicated an association between oral and systemic bone. More recent studies have included larger numbers of women with a wide range of bone mineral density in systemic bone. Wactawski-Wende et al. (1996) [183] showed positive correlations between alveolar bone loss and bone mineral density at the spine, trochanter, Ward's triangle or total femur. Further, cross-sectional data from 468 postmenopausal females enrolled in the oral ancillary portion of the Women's Health Initiative study revealed a significant correlation between basal bone density determined from intraoral radiographs and hip bone mineral density determined by DXA [79].

On the other hand, while some studies indicate osteoporosis as a risk indicator for periodontitis [153, 176], others have not detected a significant association [187]. Moreover, there is only a limited number of longitudinal studies evaluating the association of osteoporosis and periodontitis progression. Reinhardt et al. (1999) [147] prospectively analyzed the influence of serum estradiol levels and osteopenia / osteoporosis on common clinical measurements of periodontal disease over a 2-year period. No significant differences were found in attachment loss between osteoporotic and non-osteoporotic patients, although the authors reported a trend towards more attachment loss in non-smoking osteoporotic patients. In contrast, in a recent longitudinal study of 184 individuals aged 70 years [190], bone mineral density was associated with the number of progressive sites which had ≥ 3 mm additional attachment loss over 3 years, suggesting a significant relationship between periodontal disease and general bone mineral density.

It may therefore be concluded that the relationship between osteoporosis and periodontitis remains unclear. Confounding factors such as age, gender or smoking and the lack of precise methods for the assessment of osteoporosis in the jaws have been reported to

affect the establishment of a clear interaction between osteoporosis and periodontitis. Larger prospective longitudinal studies are needed to further evaluate osteoporosis as a risk factor for progressive periodontitis.

Genetic Factors

While microbial and other environmental factors are believed to initiate and modulate periodontal disease progression, there now exists strong supporting evidence that genes play a role in the predisposition to and progression of periodontal diseases [70, 73]. Support for this statement comes from studies in animals and humans which indicate that genetic factors influence the inflammatory and immune response in general, and periodontitis experience specifically. Different forms of genes (allelic variants) can produce variations in tissue structure (innate immunity), antibody responses (adaptative immunity) and inflammatory mediators (non-specific inflammation)[84]. Thus, complex diseases such as periodontitis may have multiple gene associations which individually have weak effects but which collectively combine with other influences, such as environmental factors, and result in various disease manifestations [120].

The hypothesis that genetic factors account for variation in phenotype expression of periodontal disease has been formally tested by comparing disease characteristics in monozygous and dizygous twins. It is assumed in these experiments that because a given set of adult twins grew up together in the same environment there is reason to believe that they should share most relevant habits and practices. Thus the similarity of such factors as personal habits, lifestyles and access to health care should not be different for members of twin pairs whether they are monozygous or dizygous. Michalowicz et al. (1991) [113, 114], in studies of both monozygous and dizygous twins reared together and apart, showed a significant genetic component for probing depth, attachment loss and radiographic alveolar bone height, supporting the role of genetics in periodontal disease. In a recent study, Michalowicz et al. (2000) [115] found that monozygous twins were found to be more similar than dizygous ones for clinical measurements such as probing depth, attachment loss, plaque and gingivitis.A statistically significant genetic variance was found for both severity and extent of the disease. Based on this study, chronic periodontitis was estimated to have approximately 50% heritability, which was unaltered following adjustments for behavioral variables including smoking. However, while monozygous twins were also more similar than dizygous twins for gingivitis scores, there was no evidence of heritability for gingivitis after behavioral covariates such as utilization of dental care and smoking were incorporated into the analyses. In short, these studies indicate that approximately half of the variance in chronic periodontitis in the population is attributed to genetic variation. Thus the basis of heritability of periodontitis seems to be biological and not behavioral.

Interest in identifying genetic risk factors for chronic periodontal diseases has been spurred by recent reports of associations with polymorphisms. Gene polymorphisms are locations within the genome that vary in sequence between individuals and are very prevalent, affecting at least 1% of the population. The rationale for studying single gene nucleotide polymorphisms is that they can be used to identify potential markers of susceptibility,

severity and clinical outcome [84]. Various aspects of the host inflammatory response have attracted attention as potentially crucial variants influencing the host response in periodontitis.

Cytokines

Specific genotypes have been identified and linked to periodontal destruction. Polymorphisms of interleukin-1 (IL-1), IL-1β and IL-1RN genotypes have been identified as potential risk factors for periodontal destruction. In 1997, Kornman et al. [86] were the first to describe an association between polymorphisms and periodontal disease, creating a great interest in the topic. They found an association between polymorphisms in the gene encoding for IL-1α (-889) and IL-1β (+3953) and an increased severity of periodontitis. Functionally, IL-1 genotype is associated with high levels of IL-1 production [141]. A role has been suggested for IL-1 in the initiation and progression of periodontitis. IL-1 may activate the degradation of the extracellular matrix and bone of the periodontal tissues, and increased tissue or gingival fluid levels of IL-1β have been associated with periodontitis [84]. Moreover, it was found that the mean counts of specific bacteria species were higher in IL-1 genotype-positive individuals than in negative subjects [167]. Several studies have corroborated the association between IL-1 polymorphism and periodontal disease or tooth loss [39, 109, 184]. Furthermore, a recent systematic review and meta-analysis established a statistically significant association of IL-1A (-889) and IL-1B (+3953) polymorphisms with chronic periodontal disease [121]. However, Huynh-Ba et al. (2007) [75] in a previous systematic review suggested that there is insufficient evidence to establish whether a positive IL-1 genotype status contributes to the progression of periodontitis and/or treatment outcomes. The data thus remain inconclusive, and longitudinal studies are required to establish the extent to which this genetic factor plays a role in disease progression.

The polymorphism of tumor necrosis factor-α (TNF-α) has also been suggested as a possible risk factor for periodontitis. TNF-α is secreted as a response to bacterial stimulation by a variety of cell types [173]. It stimulates osteoclasts differentiation and together with IL-1 may result in bone resorption [103]. Furthermore, TNF-α promotes the release of collagenases (metalloproteinase) that destroy the extracellular matrix [21] and are produced in excessive amounts in inflamed periodontal tissues [66]. However, most studies have failed to link this polymorphism of the TNF-α gene to chronic periodontitis [36, 59, 121].

Human Leukocyte Antigens

Several investigations have studied populations of patients with different forms of periodontitis to determine the expression of polymorphisms of human leukocyte antigens (HLA). The HLA complex plays an important role in immune responsiveness and may be involved in antigen recognition of periodontal pathogens. Recognition of antigen peptides and their presentation to T cells is crucial for an effective antigen-specific immune response to periodontal pathogens and underlies genetic control. Because antigen presentation to and resultant activation of T cells is restricted by the major histocompatibility complex (MHC), the polymorphism of the human MHC molecules (human leukocyte antigens – HLA) may

directly affect the binding capability of antigen peptides and thus the antigen-specific T-cell response [192]. A recent systematic review did not reveal any significant positive or negative associations [170]. However, few studies are available and those present significant limitations, such as the control group not always being healthy. On the other hand, when aggressive periodontitis was evaluated, an association with particular HLA polymorphisms was observed. Therefore, more studies are needed before definite conclusions can be drawn.

Immuno-Receptors

The association of immuno-receptors with periodontitis has been studied. Receptors for Fc domain of IgG FCγR) are categorized as a family of receptors, expressed on the cell surface of leukocytes, which bind IgG antibodies and immune complexes [102]. In humans, FCγRs are expressed on natural killer cells, macrophages, T lymphocytes, monocytes and mast cells [65]. The interaction between FCγRs and IgG triggers a variety of biological responses, including phagocytosis, endocytosis, antibody-dependent cellular cytotoxicity, release of inflammatory mediators, and enhancement of antigen presentation [84]. Polymorphisms that influence the binding affinity between FCγR and IgG of different subclasses are considered important in susceptibility to periodontal diseases. The few existing studies of chronic periodontitis have investigated associations between FCγR polymorphisms and susceptibility to and severity of periodontitis. The majority of them indicate that polymorphisms of FCγR tend to be associated with the chronic form of peridontitis [31, 85, 112].

Matrix Metalloproteinases

Matrix metalloproteinases (MMPs) are one of the most important groups of enzymes involved in periodontal connective tissue destruction [169]. Although few studies have suggested an association between MMP gene polymorphisms and chronic periodontitis [140, 169], there is strong controversial evidence for such an association. Itagaki et al. (2004) [140] reported that MMP-1 and / or MMP-3 single nucleotide polymorphisms were not associated with susceptibility to periodontitis in a Japanese population. More recently, polymorphisms in the gene for MMP-2 were studied and no definite correlation with periodontitis could be found [74]. Repeke et al. (2009) [150] observed a limited role for MMP-1 polymorphism in periodontitis. It seems that the extensive chronic antigenic challenge exposure overcomes the genetic control and plays a major role in the determination of MMP-1 expression. Therefore, due to the limited number of studies carried out to date, it is difficult to associate single nucleotide polymorphisms of MMP genes with chronic periodontitis.

Reports on the genetic polymorphisms associated with chronic periodontal diseases are increasing, encouraging the search for new specific markers by researchers, but the limitations of such studies have not been fully appreciated. For example, in nearly all the published studies, subjects have not been characterized as to behavioral risk factors such as smoking, stress or others. In addition, the heterogeneity of the diseases examined and the ethnic aspects of the distribution of the genetic markers may contribute to the disparity of the

results [77]. In conclusion, some gene polymorphisms are associated with modest increases in the probability of periodontal disease developing. Further studies on the distribution and dynamics of genetic variation at many loci simultaneously might disclose the direct and epistatic (interaction among multiple alleles) genetic involvement in periodontitis.

Microbial Composition

While periodontal disease is regarded as an opportunistic mixed microbial infection, specific periodontal pathogens have been proposed as predictors for further disease progression[72]. Although there are over 500 different intra-oral species and other that have not yet been identified, the majority of studies have focused on a subset of microorganisms including Agreggatibacter actinomycetencomitans (A.a.), Porphyromonas gingivalis (P.g.) and Tannerella forsythia (T.f.) [55, 126], presumably because they satisfy the criteria for Socransky's modifications of Koch's postulates:

- the organism must occur at higher numbers in disease-active sites than disease-inactive sites;
- elimination of the organism should arrest disease progression;
- the organism should elicit a humoral or cellular immune response;
- animal pathogenicity testing should infer disease potential;
- the organism should possess virulence factors relevant to the disease process;

Regarding the virulence factors, A.a., P.g. and T.f. share three common features that support their role as risk factors for initiation and progression of periodontitis. First, all are Gram-negative, and therefore produce lipopolyssacharide, which can modulate the local inflammatory response in host cells that express pattern recognition receptors. Moreover, all appear capable of invasion of the mucosal barrier to infection and possibly of being sequestered inside epithelial cells. And finally, all produce factors that enable them to evade the antibacterial functions of the innate immune response either passively (anti-phagocytic capsule) or actively (leukotoxin, gingipains, proteases, induction of apoptosis) [55].

However, evaluation of these three pathogens as risk factors for attachment loss over time has resulted in conflicting evidence. Some studies do not support the detection of these specific bacterial species for the identification of individuals at risk for periodontitis progression [98, 104]. On the other hand, a number of longitudinal studies have shown that the presence of high levels of these species at baseline is a prognostic indicator for disease progression [68, 106, 177]. Individually, A.a. has been implicated only in some cases of chronic periodontitis [24, 152, 177]. Its association has been most clearly demonstrated with localized aggressive periodontitis [71]. On the other hand, the importance of P.g. and T.f. in the initiation of chronic periodontitis as well as in its progression to advanced periodontitis is more clearly established in longitudinal studies [106, 180]. Further evidence suggests that B.f. and presumably P.g. are also associated with disease recurrence when patients are followed up after therapy [29].

Although this review has focused on the three bacterial species considered most likely to initiate the events resulting in chronic periodontitis, there are several other microorganisms

that have been described as moderately associated with the disease. These species include Campylobacter rectus, Eubacterium nodatum, Fusobacterium nucleatum, Prevotella intermedia, Peptostreptococcus micros, Streptococcus intermedius-complex and Treponema denticola [127].

The evidence for the prognostic value of A.a., P.g. and T.f. remains inconclusive, and the role of the other pathogenic bacteria has likewise yet to be fully appreciated. Such evidences, however, does lead us to believe that certain bacteria like P.g. and T.f. are indeed more important than others when it comes to considering risk indicators of chronic periodontitis.

Tooth-Level Factors

Individual variation in susceptibility to disease progression may be related to a number of a local clinical factors including tooth position [2], caries and defective restoration margins [3, 22], subgingival restoration margins [163], abutment tooth [145], presence of calculus [119], occlusal discrepancies [125], unsatisfactory root form [109] or root grooves [93].

A number of periodontal parameters have also been shown to influence periodontitis progression: gingivitis/bleeding on probing [43, 92], probing depth [13, 33], alveolar bone loss [145], tooth mobility [56], furcation involvement [41] and tooth type [118].

In particular, bleeding on probing, pocket depth and radiographic alveolar bone loss are considered to be of great importance by the clinicians for decision making [136]. But do these factors really predict future attachment loss?

Current theory holds that the gingival lesion is the precursor of periodontitis. Clearly, not all gingivitis lesions progress to periodontitis. It has been suggested that individuals are at lower risk for disease progression if the prevalence of bleeding on probing at a subject level is $\leq 25\%$ [81]. However, the proportion of gingival lesions progressing to periodontitis and the factors causing this conversion have not yet been sufficiently clarified. Periodontitis and mean attachment loss have been positively associated with bleeding on probing [43]. Recently, a longitudinal study of a patient cohort of 565 males was performed over a 26-year period. Sites with consistent bleeding had 70% more attachment loss than sites that were consistently non-inflamed. Moreover, teeth with sites that were consistently non-inflamed had a 50-year survival rate of 99.5%, while teeth with consistently inflamed gingivae yielded a 50-year survival rate of 63.4% [92].

Regarding pocket depth, on a site basis, the presence of deep residual pockets has been associated with disease progression [13, 33]. A systematic review addressing the use of residual pocket depth, bleeding on probing and furcation status following initial periodontal therapy to predict further attachment and tooth loss found that, at the individual level, residual pocket depth was predictive of further disease progression [149].

Furthermore, longitudinal studies of periodontal disease have shown that the amount of alveolar bone loss present at baseline, which represents the patient's previous history of periodontitis, may be also used to predict further progression of untreated and treated periodontitis [56, 133, 145].

Despite the importance of clinical findings on the progression of periodontal disease, treatment planning based only on the assessment of disease severity rather than other documented risk factors such as environmental and systemic factors leaves much to be desired [136].

Multifactorial Risk Assessment Models

The management of periodontal disease patients is used to be based on a "repair" model of care, in which clinician's goal was to diagnose the problem and resolve it via treatment. In recent years, however, an increasing understanding of the aetiology and risk factors for chronic periodontal diseases has developed. As a result, their management is undergoing a transition from a repair model to the wellness model of patient care that guides the clinician toward a health care strategy based on risk reduction and disease prevention [130]. Rather than the mere application of the knowledge of the risk factors to maintain oral health and to prevent the onset of periodontal disease, attention has been drawn to the assessment of risk level for disease progression in individuals under supportive periodontal therapy, representing a population with a moderate to high level of tisk of periodontal breakdown has attracted attention. The assessment of risk level for disease progression in each individual patient would enable the practitioner to determine the frequency and extent of professional support necessary to maintain the attachment levels obtained following active therapy [91]. Moreover, the clinician often has to decide which teeth to retain, which treatment to prescribe, or how to maintain or restore a functional and aesthetically pleasing dentition. For decision making at a tooth level, it is of paramount importance to assess prognosis of each tooth in order to choose the treatment modality with the greatest probability of success [56].

Thus, as the study of prognostic factors has progressed, multi-factorial risk assessment modelshas been proposed using the combination of these factors to identify individuals and teeth at high risk for periodontitis progression [56, 91, 130, 136, 149].

Periodontal Risk Calculator (PRC) (Page et al., 2002)

Page et al. (2002) [130] developed a computer-based tool, the periodontal risk calculator (PRC), for assessing risk and predicting periodontal deterioration. The PRC is based on a mathematically derived algorithms that assign relative weights to various known risks that increase patients' susceptibility to develop periodontitis: patient age, smoking history, diabetes diagnosis, history of periodontal surgery, pocket depth, bleeding on probing, restorations below the gingival margin, root calculus, radiographic bone height, furcation involvements and vertical bone lesions. The aim of the PRC is to be user-friendly and to require only information that is gathered during a routine periodontal examination. The PRC determines the patient's level of risk on a scale from 1 (lowest) to 5 (highest). However, the details of the algorithm and weighting for the factors have not been published.

Page et al. (2003) [131] documented the extent of agreement between risk scores calculated using the PRC and information gathered during a baseline examination with the periodontal status 3, 9 and 15 years later. In a retrospective study, clinical records and radiographs of 523 men were used. Information from baseline examinations was entered into the risk calculator and a risk score on a scale of 1-5 for periodontal deterioration was calculated for each subject. Actual periodontal status in terms of alveolar bone loss determined using digital radiographs, and tooth loss determined from the clinical records, was assessed at 3, 9, and 15 years. The risk scores at baseline were found to be strong predictors of future periodontal status measured as worsening severity and extent of alveolar bone loss

and tooth loss, especially loss of periodontally affected teeth. The authors concluded that risk scores calculated using the PRC and information gathered during a standard periodontal examination predict future periodontal status with a high level of accuracy and validity.

Periodontal Risk Assessment (PRA) (Lang & Tonetti, 2003)

Lang & Tonetti (2003) [91] constructed a functional diagram to assess patient's risk of recurrence of periodontitis based on a number of risk factors and risk indicators evaluated simultaneously. The PRA model consists of an assessment of the proportion of bleeding on probing, the prevalence of residual pockets greater than 4 mm (≥ 5 mm), the tooth loss from a total of 28 teeth, the loss of periodontal support (proportion of sites with bleeding on probing) in relation to the patient's age, the systemic and genetic condition (e.g. diabetes mellitus and polymorphism of interleukin-1, respectively), and environmental factors, such as cigarette smoking. Each parameter has its own scale for minor, moderate and high risk profiles (Figure 1).

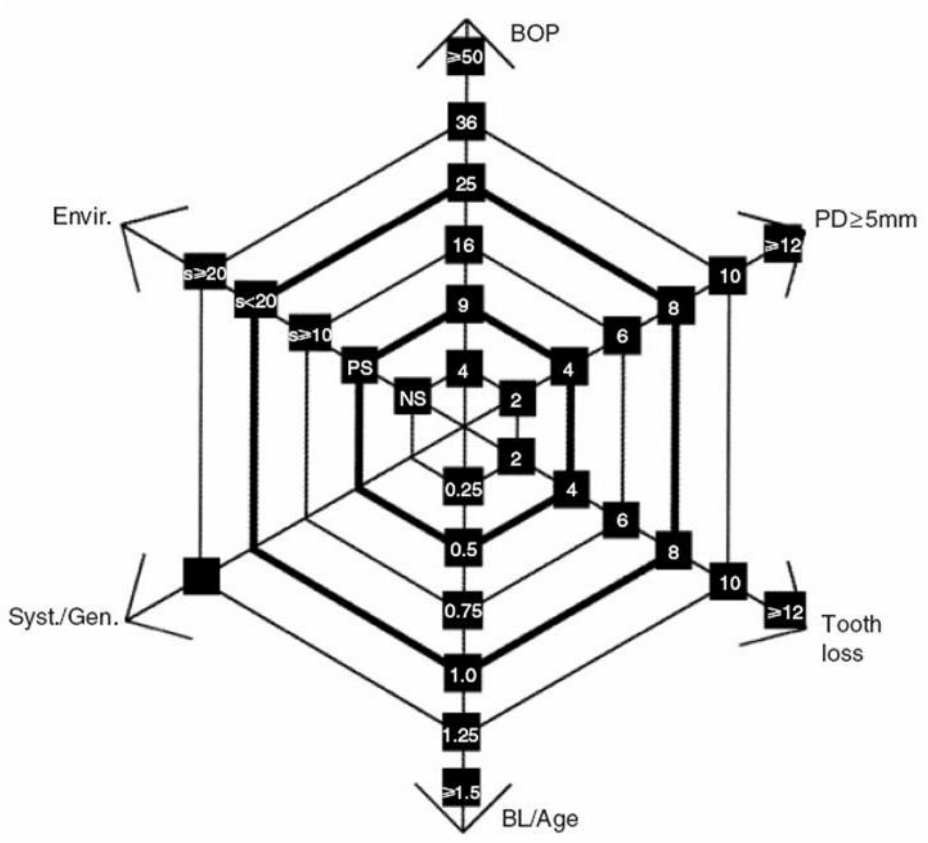

Figure 1. Schematic illustration representing a periodontal risk assessment functional diagram. Each vector represents a single risk factor or indicator. The area of low risk is found within the centre circle of the polygon, while the area of high risk lies outside the periphery of the second ring in bold. Between the two rings in bold is the area of moderate risk (Lang & Tonetti, 2003).

The authors provided evidence supporting the inclusion of each parameter within the diagram. The hexagonal risk diagram identified patients at low risk (all parameters within the low risk categories or, at the most, one parameter in the moderate) and those at moderate (at least two parameters in the moderate category, but at most one parameter in the high risk) and high risk (at least two parameters in the high risk category). Thus, a comprehensive evaluation of the functional diagram would provide an individualized total risk profile and determine the frequency and complexity of supportive periodontal therapy visits. However, this model was not validated and little evidence on its applicability is available. In a retrospective study including 100 patients who had received active treatment, Eickholz et al. (2008) [52] were the first to provide evidence that patients assigned to the high risk group according to the Lang & Tonetti risk assessment suffered from a higher rate of tooth loss after a 10-year follow-up than the other risk groups.

PRA / Multifactorial Risk Diagram (Renvert &Persson, 2004)

In this multifactorial risk diagram, a modification of the PRA model is described where the vector bone loss index (bone loss in relation to subject's age) is replaced by the proportion of sites with a distance ≥ 4 mm from the cementoenamel junction to the bone level [149]. The individuals were not more categorized as low, moderate or high risk. Here, the surface outlined between the various risk parameters was calculated to provide a numerical score of risk with the aid of a computer program (EXCEL XP for PC, Redmond, WA, USA). The authors suggested that in this way the risk scores can be monitored and compared over time, enabling the clinician to adjust the supportive therapy strategy as appropriate.

Prognostic Model for Tooth Survival (Faggion et al., 2007)

Faggion et al. (2007) [56] developed a prognostic model to estimate quantitatively survival rates for teeth in patients receiving treatment for periodontitis, in order to make evidence-based decisions about retaining or extracting teeth. With the aim of constructing the prognostic model, one hundred and ninety-eight patients were included in a retrospective study. At baseline, medical history (diabetes mellitus, coronary heart diseases, infectious diseases, allergies, coagulation disorders and radiation in the head and neck regions), clinical findings (teeth present, caries, dental restorations, probing depth, tooth mobility, approximal plaque index, sulcus bleeding test and tooth vitality) and full-mouth radiographs (alveolar bone level) were available. A logistic regression model revealed the following significant predictors for tooth loss during supportive periodontal therapy: a diagnosis of diabetes mellitus, the alveolar bone level, tooth mobility, root type and non-vital pulp at baseline examination. Based on these parameters, a prognostic model was constructed that provides estimates of tooth survival probability when periodontal therapy was performed (Figure 2). The authors showed that prognosis of tooth loss improved 14%, as compared with an alternative prognosis that did not consider any information provided by prognostic variables.

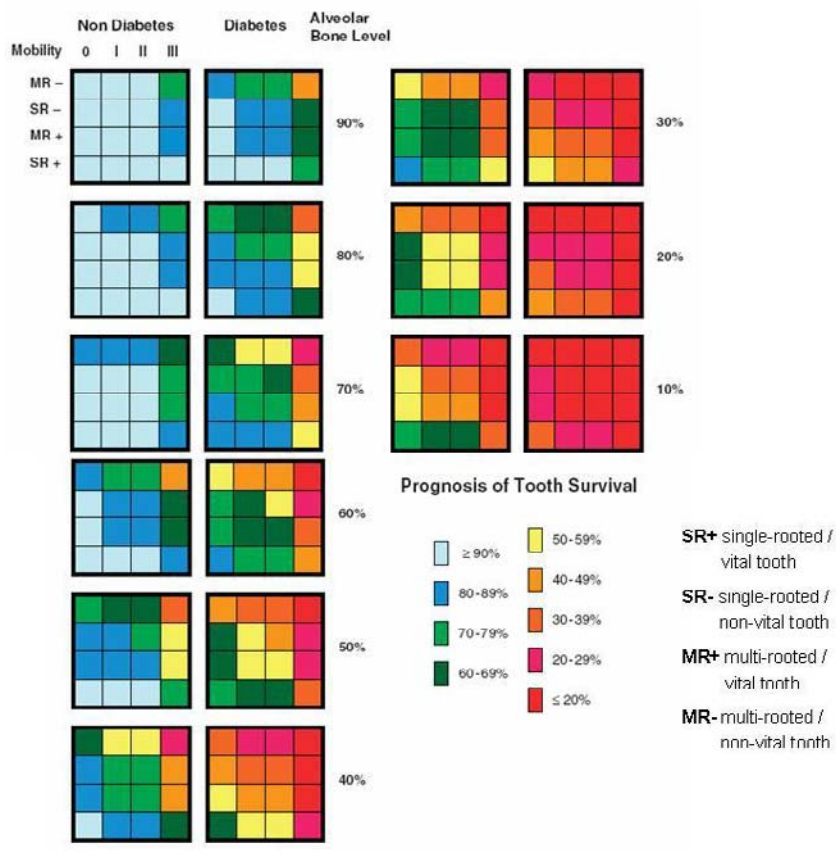

Figure 2. Schematic illustration representing a prognostic model for tooth survival. Each square represents a unique combination of predictors and the color coding on the bottom right indicates the likelihood of tooth survival probability (Faggion et al., 2007).

Conclusion

The above review clearly shows that chronic periodontal diseases are multifactorial disorders. Microbial dental plaque biofilm is the principal etiological factor, although several other local and systemic factors play an important modifying role in their pathogenesis. There is overwhelming evidence that both smoking and diabetes are important risk factors for periodontal tissue loss. In addition, the role of genetic factors and emotional stress has recently been highlighted. However, there is still a need for further studies to establish with great precision the contributions of other factors in the pathogenesis of these diseases.

Multifactorial risk models based on a knowledge of risk factors and risk indicators have been proposed to enhance the ability to predict risk for periodontal disease progression. However, prospective studies are virtually nonexistent to date. Moreover, few host-related factors are included in these models which may account for perhaps explain their limited improvement in predicting future disease events. Research in this field should be encouraged with the ultimate goal of helping the decision making during treatment planning and also to guide the clinician toward a strategy of risk reduction and disease prevention.

References

[1] Abdellatif HM, Burt BA. *An epidemiological investigation into the relative importance of age and oral hygiene status as determinants of periodontitis*. J Dent Res 1987;66:13-18.

[2] Ainamo J. *Relationship between malalignment of the teeth and periodontal disease.* Scand J Dent Res 1972;2:104-110.

[3] Albandar JM, Buischi YA, Axelsson P. *Caries lesions and dental restorations as predisposing factors in the progression of periodontal diseases in adolescents. A 3-year longitudinal study.* J Periodontol 1995;4:249-254.

[4] Albandar JM, Kingman A, Brown LJ, Löe H. *Gingival inflammation and subgingival calculus as determinants of disease progression in early-onset periodontitis*. J Clin Periodontol 1998;25:231-237.

[5] Albandar JM, Kingman A. *Gingival recession, gingival bleeding, and dental calculus in adults 30 years of age and older in the United States, 1988-1994*. J Periodontol 1999a;70(1):30-43.

[6] Albandar JM, Brunelle JA, Kingman A. *Destructive periodontal disease in adults 30 years of age and older in the United States, 1988-1994*. J Periodontol 1999b;70:13-29.

[7] Albandar JM, Tinoco EM. *Global epidemiology of periodontal diseases in children and young persons.* Periodntol 2000 2002a;29:153-76.

[8] Albandar JM. *Global risk factors and risk indicators for periodontal diseases*. Periodontol 2000 2002b;29:177-206.

[9] Albandar JM. *Periodontal diseases in North America*. Periodontol 2000 2002c;29:31-69.

[10] Albandar JM. *Epidemiology and risk factors of periodontal diseases*. Dent Clin N Am 2005;49:517-532.

[11] Arbes SJ Jr, Agustsdottir H, Slade GD. *Environmental tobacco smoke and periodontal disease in the United State*s. Am J Public Health 2001;91:253-257.

[12] Armitage GC. *Development of a classification system for periodontal diseases and conditions.* Ann Periodontol 1999;4:1-6.

[13] Badersten A, Nilveus R, Egelberg J. *Scores of plaque, bleeding, suppuration and probing depth to predict probing attachment loss. 5 years of observation following non-surgical periodontal therapy.* J Clin Periodontol 1990;2:102-107.

[14] Benatti BB, Silvério KG, Casati MZ, Sallum EA, Nociti FH Jr. *Influence of aging on biological properties of periodontal ligament cells*. Connect Tissue Res 2008;49:401-408.

[15] Benatti BB, Silvério KG, Casati MZ, Sallum EA, Nociti FH Jr. *Inflammatory and bone-related genes are modulated by aging in human periodontal ligament cells*. Cytokine 2009;46:176-181.

[16] Bergström J, Eliasson S, Preber H. *Cigarette smoking and periodontal bone loss.*J Periodontol 1991;62:242-246.

[17] Bergström J. *Tobacco smoking and supragingival dental calculus*. J Clin Periodontol 1999;26:541-547.

[18] Bergström J, Bostrom L. *Tobacco smoking and periodontal hemorrhagic responsiveness.* J Clin Periodontol 2001;28:680-685.

[19] Bergström J. *Tobacco smoking and risk for periodontal disease*. J Clin Periodontol 2003;30:107-113.

[20] Boyapati L, Wang H-L. *The role of stress in periodontal disease and wound healing.* Periodontol 2000 2007;44:195-210.

[21] Brenner DA, O'Hara M, Angel P, Chojkier M, Karin M. *Prolonged activation of jun and collagenase genes by tumour necrosis factor-alpha.* Nature 1989;337:661-663.

[22] Broadbent JM, Williams KB, Thomson WM, Williams SM. *Dental restorations: a risk factor for periodontal attachment loss?* J Clin Periodontol 2006;33:803-810.

[23] Brown LJ, Oliver RC, Löe H. *Evaluating periodontal status of U.S. employed adults*. J Am Dent Assoc 1990;121:226-232.

[24] Buchmann R, Muller RF, Heinecke A, Lange DE. *Actinobacillus actinomycetencomitans in destructive periodontal disease. Three-year follow-up results.* J Periodontol 2000;71:444-453.

[25] Calsina G, Ramon JM, Echeverria JJ. *Effects of smoking on periodontal tissues*. J Clin Periodontol 2002;29:771-776.

[26] César-Neto JB, Duarte PM, Sallum EA, Barbieri D, Moreno H Jr, Nociti FH Jr. *A comparative study on the effect of nicotine administration and cigarette smoke inhalation on bone healing around titanium implants*. J Periodontol2003;74:1454-1459.

[27] César-Neto JB, Benatti BB, Haiter-Neto F, Sallum AW, Sallum EA, Nociti FH Jr. *Smoking cessation may present a positive impact on mandibular bone quality and periodontitis-related bone loss: a study in rats*. J Periodontol 2005;76:520-525.

[28] César-Neto JB, Duarte PM, de Oliveira MCG, Tambelli CH, Sallum EA, Nociti FH Jr. *Smoking modulates interleukin-6:interleukin-10 and RANKL:osteoprotegerin ratios in the periodontal tissues*. J Periodont Res 2007;42:184-191.

[29] Chaves ES, Jeffcoat MK, Ryerson CC, Snyder B. *Persistent bacterial colonization of Porphyromonas gingivalis, Prevotella intermedia, and Actinobacillus actinomycetencomitans in periodontitis and its association with alveolar bone loss after 6 months of therapy.* J Clin Periodontol 2000;27:897-903.

[30] Christgau M, Palitzsch KD, Schmalz G, Kreiner U, Frenzel S. *Healing response to non-surgical periodontal therapy in patients with diabetes mellitus: clinical, microbiological, and immunologic* results. J Clin Perioodntol 1998;2:112-124.

[31] Chung H-Y, Lu H-C, Chen W-L, Lu C-T, Yang Y-H, Tsai C-C. *Gm (23) allotypes and Fcγ receptor genotypes as risk factors for various forms of periodontitis*. J Clin Periodontol 2003;30:954-960.

[32] Ciancio SG. *Current status of indices of gingivitis*. J Clin Periodontol 1986;13:375-82.

[33] Claffey N, Nylund K, Kiger R, Garrett S, Egelberg J. *Diagnostic predictability of scores of plaque, bleeding, suppuration and probing depth for probing attachment loss. 3 ½ years of observation following initial periodontal therapy*. J Clin Periodontol 1990;2:108-114.

[34] Consensus Report: chronic periodontitis. Ann Periodontol 1999;4:38.

[35] Corberand J, Laharrague P, Nguyen F, Duat G, Fontanilles M, Gleizes B, Gyrard E. *In vitro effect of tobacco smoke components on the functions of normal human polymorphonuclear leukocytes.* Infect Immun 1980;30:649-655.

[36] Craandijk J, van Krugten MV, Verweij CL, van der Velden U, Loos BG. *Tumor necrosis factor-alpha gene polymorphisms in relation to periodontitis.*J Clin Periodontol 2002;29:28-34.

[37] Craig RG, Boylan R, Yip J, Bamgboye P, Koutsoukos J, Mijares D, Ferrer J, Imam M, Socransky SS, Haffajee AD. *Prevalence and risk indicators for destructive periodontal diseases in 3 urban American minority populations*. J Clin Periodontol 2001;6:524-535.

[38] Croiset G, Heijinen C, Wied D. *Passive avoidance behavior, vasopressin and immune system: a link between avoidance latency and immune response*. Neuroendocrinology 1990;51:156-161.

[39] Cullinan MP, Westerman B, Hamlet SM, Palmer JE, Faddy MJ, Lang NP, Seymour GP. *A longitudinal study of interleukin-1 gene polymorphisms and periodontal disease in a general adult population*. J Clin Periodontol 2001;28:1137-1144.

[40] Dakovick D, Pavlovic MD. *Periodontal disease in children and adolescents with type 1 diabetes in Servia*. J Periodontol 2008;79:987-992.

[41] Dannewitz B, Krieger JK, Hüsing J, Eickholz P. *Loss of molars in periodontally treated patients: a retrospective analysis five years of more after active periodontal treatment*. J Clin Periodontol 2006;33:53-61.

[42] Dietrich T, Bernimoulin JP, Glynn RJ. *The effect of cigarette smoking on gingival bleeding*. J Periodontol 2004;75:16-22.

[43] Dietrich T, Kaye EK, Nunn ME, Van Dyke T, Garcia RI. *Gingivitis susceptibility and its relation to periodontitis in men.* J Dent Res 2006;85:1134-7.

[44] Dolan TA, Gilbert GH, Ringelberg ML, Legler DW, Antonson DE, Foerster U, Heft MW. *Behavioral risk indicators of attachment loss in adult Floridians*. J Clin Periodontol 1997;24:223-232.

[45] Dorland. *Dorland's illustrated medical dictionary*. Oxford, UK:WB Sauders, 2000.

[46] Drury TF, Garcia I, Adesanya M. *Socioeconomic disparities in adult oral health in the United States*Ann NY Acad Sci 1999;896:322-324.

[47] Duarte PM, Assis DR, Casati MZ, Sallum AW, Sallum EA, Nociti FH Jr. *Alendronate may protect against increased periodontitis-related bone loss in estrogen-deficient rats*. J Periodontol 2004a;75:1196-1202.

[48] Duarte PM, Gonçalves PF, Sallum AW, Sallum EA, Casati MZ, Nociti FH Jr *Effect of an estrogen-deficient state and its therapy on bone loss resulting from an experimental periodontitis in rats.* J Periodontol 2004b;39:107-110.

[49] Duarte PM, Gonçalves PF, Casati MZ, de Toledo S, Sallum EA, Nociti FH Jr. *Estrogen and alendronate therapies may prevent the influence of estrogen deficiency on the tooth-supporting alveolar bone: a histometric stuady in rats.* J Periodont Res 2006;41:541-546.

[50] Duarte PM, de Oliveira MCG, Tambelli CH, Parada CA, Casati MZ, Nociti FH Jr. *Overexpression of interleukin-1β and interleukin-6 may play an important role in periodontal breakdown in type 2 diabetic patients*. J Periodont Res 2007;42:377-381.

[51] Eggert FM, McLeod MH, Flowerdew G. *Effects of smoking and treatment status on periodontal bacteria: evidence that smoking influences control of periodontal bacteria at the mucosal surface of the gingival crevice.* J Periodontol 2001;72:1210-1220.

[52] Eickholz P, Kaltschmitt J, Berbig J, Reitmeir P, Pretzl B. *Tooth loss after active periodontal therapy. 1: patient-related factors for risk, prognosis, and quality outcome*. J Clin Periodontol 2008;35:165-74.

[53] Emrich LJ, Shlossman M, Genco RJ. *Periodontal disease in non-insulin-dependent diabetes mellitus.* J Periodontol 1991;2:123-131.

[54] Eriksen EF, Colvard DS, Berg NJ, Graham ML, Mann KG, Spelsberg TC, Riggs BL. *Evidence of estrogen receptors in normal human osteoblast-like cells*. Science 1988;241:84-86.

[55] Ezzo PJ, Cutler CW. *Microorganisms as risk indicators for periodontal disease.* Periodontol 2000 2003;32:24-35.

[56] Faggion CM Jr, Petersilka G, Lange DE, Gerss J, Flemmig TF. *Prognostic model for tooth survival in patients treated for periodontitis.* J Clin Periodontol. 2007;34:226-31.

[57] Franceschi C, Bonafè M, Valensin S, Olivieri F, de Luca M, Ottaviani E, De Benedictis G. *Inflamm-aging. An evolutionary perspective on immunosenescence*. Ann NY Acad Sci 2000;908:244-54.

[58] Fredriksson MI, Figueredo CM, Gustafsson A, Bergström KG, Asman BE. *Effects of periodontitis and smoking on blood leukocytes and acute-phase proteins*. J Periodontol 1999;70:1355-1360.

[59] Galbraith GM, Steed RB, Sanders JJ, Pandey JP. *Tumor necrosis factor alpha production by oral leukocytes: influence of tumor necrosis factor genotype*. J Periodontol 1998;69:428-433.

[60] Gemmell E, Seymour GJ. *Immunoregulatory control of Th1/Th2 cytokine profiles in periodontal disease*. Periodontol 2000 2004;35:21-41.

[61] Geurs NC. *Osteoporosis and periodontal disease*. Periodontol 2000 2007;44:29-43.

[62] Giddon DB, Zackin SJ, Goldhaber P. *Acute necrotizing ulcerative gingivitis in college students.* J Am Dent Assoc 1964: 68:380-386.

[63] Girasole G, Passeri G, Pedrazzoni M, Giuliani N, Passeri M. Interleukin-6: *A pathogenic role in the postmenopausal osteoporosis?*Acta Biomed Ateneo Parmense 1995;66:125-138.

[64] Goldman MJ, Ross LF, Goteiner D. *Effect of periodontal therapy on patients maintained for 15 years or longer. A retrospective study*. J Periodontol 1986;6:347-353.

[65] Gonzales JR, Kobayashi T, Michel J, Mann M, Yoshie H, Meyle J. *Interleukin-4 gene polymorphisms in Japanese and Caucasian patients with aggressive periodontitis*. J Clin Periodontol 2004;31:384-389.

[66] Graves DT, Cochran D. *The contribution of interleukin-1 and tumor necrosis factor to periodontal tissue destruction*. J Periodontol 2003;74:391-401.

[67] Grupe H, Wilder L. *Observations of necrotizing gingivitis in 870 military trainees*. J Periodontol 1956;45:255-266.

[68] Haffajee AD, Socransky SS, Smith C, Dibart S. *Relation of baseline microbial parameters to future periodontal attachment loss.* J Clin Periodontol 1991;18:744-750.

[69] Haffajee AD, Socransky SS. *Relationship of cigarette smoking to attachment level profiles*. J Clin Periodontol 2001;28:283-295.

[70] Hassell TM, Harris EL. *Genetic influences in caries and periodontal disease*. Crit Rev Oral Biol Med 1995;6:319-342.

[71] Haubek D, Ennibi OK, Poulsen K, Vaeth M, Poulsen S, Kilian M. *Risk of aggressive periodontitis in adolescent carriers of the JP2 clone of Aggregatibacter (Actinobacillus) actinomycetencomitans in Morocco: a prospective longitudinal cohort study*. Lancet 2008;371:237-242.

[72] Heitz-Mayfield LJA. *Disease progression: identification of high risk groups and individuals for periodontitis.* J Clin Periodontol 2005;32(Suppl 6):196-209.

[73] Hodge P, Michalowicz B. *Gentic predisposition to periodontitis in children and young adults*. Periodontol 2000 2001;26:113-134.

[74] Holla LI, Fassman A, Vasku A, Goldbergova M, Beranek M, Znojil V, Vanek J, Vacha J. *Genetic variations in the human gelatinase a (matrix metalloproteinase-2) promoter are not associated with susceptibility to, and severity of, chronic periodontitis.*J Periodontol 2005;76:1056-1060.

[75] Huynh-Ba G, Lang NP, Tonetti MS, Salvi GE. *The association of the composite IL-1 genotype with periodontitis progression and/or treatment outcomes: a systematic review*. J Clin Periodontol 2007;34:305-317.

[76] Hyman JJ, Reid BC. *Epidemiologic risk factors for periodontal attachment loss among adults in the United States*. J Clin Periodontol 2003;30:230-237.

[77] Ioannidis JP, Trikalinos TA, Khoury MJ. *Implications of small effect sizes of individual genetic variants on the design and interpretation of genetic association studies of complex diseases*. Am J Epidemiol 2006;164:609-614.

[78] Itagaki M, Kubota T, Tai H, Shimada Y, Morozumi T, Yamazaki K. *Matrix metalloproteinase-1 and -3 gene promoter polymorphisms in Japanese patints with periodontitis.* J Clin Periodontol 2004;31:764-769.

[79] Jeffcoat MK, Lewis CE, Reddy MS, Wang C-Y, Redford M. *Post-menopausal bone loss and its relationship to oral bone loss.* Periodontol 2000 2000;23:94-102.

[80] Johnson GK, Guthmiller JM. *The impact of cigarette smoking on periodontal disease and treatment.* Periodontol 2000 2007;44:178-194.

[81] Joss A, Adler R, Lang NP. Bleeding on probing. *A parameter for monitoring periodontal conditions in clinical practice.* J Clin Periodontol 1994;6:402-408.

[82] Kanis JA, Melton LJ III, Christiansen C, JohnstonCG, Khaltaev N. *The diagnosis of osteoporosis.* J Bone Miner Res 1994;9:1137-1141.

[83] Karjalainen K, Knuuttila M. *The onset of diabetes and poor metabolic control increases gingival bleeding in children and adolescents with insulin-dependent diabetes mellitus.* J Clin Periodontol 1996;23:1060-1067.

[84] Kinane DF, Hart TC. *Genes and gene polymorphisms associated with periodontal disease*. Crit Rev Oral Biol Med 2003;14:430-449.

[85] Kobayashi T, Yamamoto K, Sugita N, van der Pol W-L, Yasuda K, Kaneko S, van der Winkel JGJ, Yoshie H. *The Fcγ Receptor genotype as a severity factor for chronic periodontitis in Japanese patients*. J Periodontol 2001;72:1324-1331.

[86] Kornman KS, Crane A, Wang Hy, di Giovine FS, Newman MG, Pirk FW, Wilson TG Jr, Higginbottom FL, Duff GW. *The interleukin-1 genotype as a severity factor in adult periodontal disease*. J Clin Periodontol 1997;24:72-77.

[87] Kribbs PJ, SmithDE, Chesnut CH.*Oral findings in osteoporosis. Part II: Relationship between residual ridge and alveolar bone resorption and generalized skeletal osteopenia.* J Prosthetic Dent 1983;50:719-724.

[88] Kribbs PJ, Chesnut CH, Ott SM, Kilcoyne RF. *Relationships between mandibular and skeletal bone in an osteoporotic population*. J Prosthetic Dent 1989;62:703-707.

[89] Kribbs PJ. *Comparison of mandibular bone in normal and osteoporotic women*. J Prosthet Dent 1990;63:218-222.

[90] Labriola A, Needleman I, Moles DR. *Systematic review of the effect of smoking on nonsurgical periodontal therapy.* Ann Periodontol 2000;5:79-89.

[91] Lang NP, Tonetti MS. *Periodontal risk assessment (PRA) for patients in supportive periodontal therapy (SPT)*. Oral Health Prev Dent 2003;1:7-16.

[92] Lang NP, Schätzle MA, Löe H. *Gingivitis as a risk factors in periodontal disease*. J Clin Periodontol 2009;36(Suppl 10):3-8.

[93] Leknes KN, Lie T, Selvig KA. *Root grooves: a risk factor in periodontal attachment loss*. J Periodontol 1994;9:859-863.

[94] Lindberg MK, Erlandsson M, Alatalo SL, Windahl S, Andersson G, Halleen JM, Carlsten H, Gustafsson JA, Ohlsson C.*Estrogen receptor alpha, but not estrogen receptor beta, is involved in the regulation of the OPG/RANKL (osteoprotegerin/ receptor activator of NF-kappa B ligand) ratio and serum interleukin-6 in male mice.* Endocrinol 2001;171:425-433.

[95] Lindhe J, Haffajee AD, Socransky SS. *Progression of periodontal disease in adult subjects in the absence of periodontal therapy*. J Clin Periodontol 1983;10:433-442.

[96] Lindhe J, Nyman S. *Long-term maintenance of patients treated for advanced periodontal disease*. J Clin Periodontol 1984;8:504-514.

[97] Lindhe J, Socransky S, Nyman S, Westfelt E, Haffajee A. *Effect of age on healing following periodontal therapy*. J Clin Periodontol 1985;12:774-787

[98] Listgarten MA, Slots J, Nowotny AH, Oler J, Rosenberg J, Gregor B, Sullivan P. *Incidence of periodontitis recurrence in treated patients with and without cultivable Actinobacillus actinomycetemcomitans, Prevotella intermedia, and Porphyromonas gingivalis: a prospective study*. J Periodontol 1991;62:377-386.

[99] Loe H, Theilade E, Jensen B. *Experimental gingivitis in man*. J Periodontol 1965;36:177-187.

[100] Loe H, Anerud A, Boysen H, Morrison E. *Natural history of periodontal disease in man. Rapid, moderate and no loss of attachment in Sri Lanka laborers 14 to 46 years of age.* J Clin Periodontol 1986;13:431-445.

[101] Loe H. Periodontal disease. *The sixth complication of diabetes mellitus*. Diabetes Care 1993;16:329-334.

[102] Loos BG, Leppers-van de Straat FG, Van de Winkel JG, Van der Velden U. *Fc gamma receptor polymorphisms in relation to periodontitis*. J Clin Periodontol 2003;30:595-602.

[103] Loos BG, John RP, Laine ML. *Identification of geneticrisk factors for parioodontitis and possible mechanisms of action*. J Clin Periodontol 2005;32(Suppl.6):159-179.

[104] MacFarlane TW, Jenkins WM, Gilmour WH, McCourtie J, McKenzie D. *Longitudinal study of untreated periodontitis (II). Microbiological findings.* J Clin Periodontol 1988;15:331-337.

[105] MacFarlane GD, Herzberg MC, Wolff LF, Hardie NA. *Refractory periodontitis associated with abnormal polymorphonuclear leukocyte phagocytosis and cigarette smoking.* J Periodontol 1992;63:908-913.

[106] Machtei EE, Dunford R, Hausmann E, Grossi SG, Powell J, Cummins D, Zambon JJ, Genco RJ. *Longitudinal study of prognostic factors in established periodontitis patients*. J Clin Periodontol 1997;2:102-109.

[107] Machtei EE, Hausmann E, Dunford R, Grossi S, Ho A, Davies G, Chandler J, Zambon J, Genco RJ. *Longitudinal studies of predictive factors for periodontal disease and tooth loss.* J Clin Periodontol 1999;6:374-380.

[108] Manoucher-Pour M, Spagnuolo PJ, Rodman HM, Bissada NF. *Comparison of neutrophil chemotactic response in diabetic patients with mild and severe periodontal disease*. J Periodontol 1981;52:410-415.

[109] McGuire MK, Nunn ME. *Prognosis versus actual outcome. IV. The effectiveness of clinical parameters and IL-1 genotype in accurately predicting prognoses and tooth survival*. J Periodontol 1999;70:49-56.

[110] McMullen JA, Van Dyke TE, Horoszewicz HU, Genco RJ. *Neutrophil chemotaxis in individuals with advanced periodontal disease and a genetic predisposition to diabetes mellitus.*J Periodontol 1981;52:167-173.

[111] Mealey BL, Ocampo GL. *Diabetes mellitus and periodontal disease*. Periodontol 2000 2007;44:127-153.

[112] Meisel P, Carlsson LE, Sawaf H, Fanghaenel J, Greinacher A, Kocher T. *Polymorphisms of Fcγ-receptors RIIa, RIIIa, and RIIIb in patients with adult periodontal diseases*. Genes Immun 2001;2:258-262.

[113] Michalowicz BS, Aeppli D, Kuba RK, Bereuter JE, Conry JP, Segal NL, Bouchard TJ Jr, Pihlstrom BL. *A twin study of genetic variation in proportional radiographic alveolar bone height.* J Dent Res 1991;70:1431-1435.

[114] Michalowicz BS, Aeppli D, Virag JG, Klump DG, Hinrichs JE, Segal NL, BouchardTJ Jr, Pihlstrom BL. *Periodontal findings in adult twins*. J Periodontol 1991;62(5):293-299.

[115] Michalowicz BS, Diehl SR, Gunsolley JC, Sparks BS, Brooks CN, Koertge TE, Califano JV, Burmeister JA, Schenkein HA. *Evidence of a substantial genetic basis for risk of adult periodontitis.* J Periodontol 2000;71:1699-1707.

[116] Miller DB, O'Callaghan JP. *Neuroendocrine aspects of the response to stress.* Metabolism 2002;51:5-10.

[117] Mullally BH, Linden GJ. *Mollar furcation involvement associated with cigarette smoking in periodontal referrals.* J Clin Periodontol 1996;23:658-661.

[118] Muzzi L, Nieri M, Cattabriga M, Rotundo R, Cairo F, Pini Prato GP. *The potential prognostic value of some periodontal factors for tooth loss: a retrospective multi-level analysis on periodontal patients treated and maintained over 10 years.*J Periodontol 2006;77:2084-2089.

[119] Neely AL, Holford TR, Loe H, Anerud A, Boysen H. *The natural history of periodontal disease in man. Risk factors for progression of attachment loss in individuals receiving no oral health care.* J Periodontol 2001;8:1006-1015.

[120] Nielsen R. *Population genetic analysis of ascertained SNP data.* Hum Genomics 2004;1:218-224.

[121] Nikolopoulos GK, Dimou NL, Hamodrakas SJ, Bagos PG. *Cytokine gene polymorphisms in periodontal disease: a meta-analysis of 53 studies including 4178 cases and 4590 controls.* J Clin Periodontol 2008;34:305-317.

[122] Nociti FH Jr, Nogueira-Filho GR, Primo MT, Machado MAN, Tramontina VA, Barros SP, Sallum EA. *The influence of nicotine on the bone loss rate in ligature-induced periodontitis. A hystometric study in rats*. J Periodontol 2000;71:1460-1464.

[123] Nociti FH Jr, Nogueira-Filho GR, Tramontina VA, Machado MAN, Barros SP, Sallum EA, Sallum AW. *Histometric evaluation of the effect of nicotine administration on periodontal breakdown.* J Periodont Res 2001;36:361-366.

[124] Novak MJ, Potter RM, Blodgett J, Ebersole J. *Periodontal disease in Hispanic Americans with type 2 diabetes*. J Periodontol 2008;79:629-636.

[125] Nunn ME, HarrelSK.*The effect of occlusal discrepancies on periodontitis. I. Relationship of initial occlusal discrepancies to initial clinical parameters*. J Periodontol 2001;4:485-494.

[126] Nunn ME. *Understanding the etiology of peridontitis: an overview of periodontal risk factors.* Periodontol 2000 2003;32:11-23.

[127] Offenbacher S, Zambon JJ. *Consensus report for periodontal diseases: pathogenesis and microbial factors.* Ann Periodontol 1996;1:926-932.

[128] Oursler MJ. *Estrogen regulation of gene expression in osteoblasts and osteoclasts*. Crit Rev Eukariot Gene Expr 1998;8:125-140.

[129] Pacifici R. *Is there a causal role for IL-1 in postmenopausal bone loss?* Calcif Tissue Int 1992;50:295-299.

[130] Page RC, Krall EA, Martin J, Mancl L, Garcia RI. *Validity and accuracy of a risk calculator in predicting periodontal disease.* J Am Dent Assoc 2002;133:569-576.

[131] Page RC, Martin J, Krall EA, Mancl L, Garcia R. *Longitudinal validation of a risk calculator for periodontal disease.* J Clin Periodontol 2003; 9:819-827.

[132] Papapanou PN, Wennström JL, Gröndahl K. *Periodontal status in relation to age and tooth type. A cross-sectional radiographic study*. J Clin Periodontol 1988;15:469-478.

[133] Papapanou EN, Wennstrom JL, Grondahl K. *A 10-year retrospective study of periodontal disease progression*. J Clin Periodontol 1989;16:403-411.

[134] Papapanou PN. World *Workshop in Clinical Periodontics. Periodontal diseases: epidemiology*. Ann Periodontol 1996;1:1-36.

[135] Paunio K. *Periodontal connective tissue. Biochemical studies of disease in man.* Suom Hammaslaak Toim 1969;65:249-290.

[136] Persson GR, Attstrom R, Lang NP, Page RC. *Perceived risk of deteriorating periodontal conditions.* J Clin Periodontol 2003;11:982-989.

[137] Peruzzo DC, Benatti BB, Ambrosano GMB, Nogueira-Filho GR, Sallum EA, Casati MZ, Nociti FH Jr. *A systematic review of stress and psychological factors as possible risk factors for periodontal disease*. J Periodontol 2007;78:1491-1504.

[138] Peruzzo DC, Benatti BB, Antunes IB, Andersen ML, Sallum EA, Casati MZ, Nociti FH Jr, Nogueira-Filho GR. *Chronic stress may modulate periodontal disease: a study in rats*. J Periodontol 2008;79:697-704.

[139] Phipps KR, Chan BKS, Jennings-Holt M, Geurs NC, Reddy MS, Lewis CE, Orwoll ES. *Periodontal health of older men: the MrOS dental study*. Gerodontology 2009;26:122-129.

[140] Pirhan D, Atilla G, Emingil G, Sorsa T, Tervahartiala T, Berdeli A. *Effect of MMP-1 promoter polymorphisms on GCF MMP-1 levels and outcome of periodontal therapy in patients with severe chronic periodontitis.* J Clin Periodontol 2008;35:862-870.

[141] Pociot F, Molvig J, Wogensen L, Worsaae H, Nerup J. *A TaqI polymorphism in the human interleukin-1 beta (IL-1 beta) gene correlates with IL-1 beta secretion in vivo.* Eur J Clin Invest 1992;22:396-402.

[142] Pontes-Andersen CC, Flyvbjerg A, Buschard K, Holmstrup P. *Relationship between periodontitis and diabetes: lessons from rodent studies.* J Periodontol 2007;78:1264-1275.

[143] Poulton R, Caspi A, Milne BJ, Thomson WM, Taylor A, Sears MR, Moffitt TE. *Association between children's experience of socioeconomic disadvantage and adult health: a life-course study*. Lancet 2002;360:1640-5.

[144] Preber H, Bergström J. *Effect of cigarette smoking on periodontal healing following surgical therapy.* J Clin Periodontol 1990;17:324-328.

[145] Pretzl B, Kaltschmitt J, kim T-S, Reitmeir P, Eickholz P. *Tooth loss after active periodontal therapy.2: tooth-related factors.* J Clin Periodontol 2008;35:175-182.

[146] Quinn SM, Zhang JB, Gunsolley JC, Schenkein HA, Tew Jg. *The influence of smoking and race on adult periodontitis and serum IgG2 levels.* J Periodontol 1998;69:171-177.

[147] Reinhardt RA, Payne JB, Maze CA, Patil KD, Gallagher SJ, Mattson JS. *Influence of estrogen and osteopenia/osteoporosis on clinical periodontitis in postmenopausal women.* J Periodontol 1999;70:823-828.

[148] Renvert S, Persson GR. *A systematic review on the use of residual probing depth, bleeding on probing and furcation status following initial periodontal therapy to predict further attachment and tooth loss.* J Clin Periodontol 2002;3:82-89.

[149] Renvert S, Persson GR. *Supportive periodontal therap*y. Periodontol 2000 2004;36:179-195.

[150] Repeke CE, Trombone APF, Ferreira SB Jr, Cardoso CR, Silveira EM, Martins W Jr, Trevilatto PC, Silva JS, Campanelli AP, Garlet GP. *Strong and persistent microbial and inflammatory stimuli overcome the genetic predisposition to higher matrix matalloproteinase -1 (MMP-1) expression: a mechanistic explanation for the lack of association of MMP1-1607 single-nucleotide polymorphism genotypes with MMP-1 expression in chronic periodontitis lesions.* J Clin Periodontol 2009;36:726-738.

[151] Riley V. *Psychoneuroendocrine influences on immunocompetence and neoplasia*. Science 1981;212:1100-1109.

[152] Rodenburg JP, van Winkelhof AJ, Winkel EG, Goené RJ, Abbas F, de Graff J. *Occurrence of Bacteroides gingivalis, Bacteroides intermedius and Actinobacillus actinomycetencomitans in severe periodontitis in relation to age and treatment history*. J Clin Periodontol 1990;17:392-399.

[153] Ronderos M, Jacobs DR, Himes JH, Pihlstrom BL. *Associations of periodontal disease with femoral bone mineral density and estrogen replacement therapy: cross-sectional evaluation of US adults from NHANES III*. J Clin Periodontol 2000;27:778-786.

[154] Rosa GM, Lucas GQ, Lucas ON. *Cigarette smoking and alveolar bone in young adults: a study using digitized radiographs.* J Periodontol 2008;79:232-244.

[155] Rosania AE, Low KG, McCormick CM, Rosania DA. *Stress, depression, cortisol and periodontal disease*. J Periodontol 2009;80:260-266.

[156] Ryder MI, Fujitaki R, Johnson G, Hyun W. A*lterations of netrophil oxidative burst by in vitro smoke exposure: implications for oral and systemic diseases.* Ann Periodontol 1998;3:76-87.

[157] Ryder MI, Saghizadeh M, Ding Y, Nguyen N, Soskolne A. *Effects of tobacco smoking on the secretion of IL-1ββ, TNF-αα and TGF-ββ from peripheral blood mononuclear cells.* Oral Microbiol Immunol 2002;17:331-336.

[158] Ryder MI. *The influence of smoking on host responses in periodontal infections.* Periodontol 2000 2007;43:267-277.

[159] Salvi GE, Yalda B, Collins JG, Jones BH, Smith FW, Arnold RR, Offenbacher S. *Inflammatory mediator response as a potential risk marker for periodontal diseases in insulin-dependent diabetes mellitus patients.* J Periodontol 1997a;68:127-135.

[160] Salvi GE, Collins JG, Yalda B, Arnold RR, Lang NP, Offenbacher S. *Monocytic TNF-α secretion patterns in IDDM patients with periodontal diseases.* J Clin Periodontol 1997b;24:8-16.

[161] Sastrowijoto SH, Hillemans P, van Steenberghe TJ, Abraham-Inpijn L, de Graff J. *Periodontal condition and microbiology of healthy and diseased periodontal pockets in type 1 diabetes mellitus patients*. J Clin Periodontol 1989;16:316-322.

[162] Sbordone L, Ramaglia L, Barone A, Ciaglia RN, Iacono VJ. *Periodontal status and subgingival microbiota of insulin-dependent juvenile diabetics: a 3-year longitudinal study*. J Periodontol 1998;69:120-128.

[163] Schätzle M, Lang NP, Anerud A, Boysen H, Burgin W, Löe H. *The influence of margins of restorations on the periodontal tissues over 26 years.* J Clin Periodontol 2001;1:57-64.

[164] Schätzle M, Löe H, Lang NP, Heitz-Mayfield LJA, Bürgin W, Anerud A, Boysen H. *Clinical course of chronic periodontitis: III. Patterns, variations and risks of attachment loss.* J Clin Periodontol 2003;30:909-918.

[165] Severson JA, Moffett BC, Kokich V, Selipsky H. *A histologic study of age changes in the adult human joint (ligament).* J Periodontol 1978;49:189-200.

[166] Shiba H, Nakanishi K, Sakata M, Fujita T, Uchida Y, Kurihara H. *Effects of ageing on proliferative ability, and the expressions of secreted protein, acidic and rich cysteine (SPARC) and osteoprotegerin (osteoclastogenesis inhibitory factor) in cultures of human periodontal ligament cells*. Mech Ageing Dev 2000;117(1-3):69-77.

[167] Socransky SS, Haffajee AD, Smith C, Duff GW. *Microbiological parameters associated with IL-1 gene polymorphisms in periodontitis patients*. J Clin Periodontol 2000;27:810-818.

[168] SoskolneWA, Klinger A. *The relationship between periodontal diseases and diabetes: an overview.* Ann Periodontol 2001;1:91-98.

[169] de Souza AP, Trevilatto PC, Scarel-Caminaga RM, Brito Jr RB, *Line SRP. MMP-1 promoter polymorphism: association with chronic periodontitis severity in a Brazilian population*. J Clin Periodontol 2003;30:154-158.

[170] Stein JM, Machulla HKG, Smeets R, lampert F, Reichert S. *Human leukocyte antigen polymorphism in chronic and aggressive periodontitis among Caucasians: a meta-analysis.*J Clin Periodontol 2008;35:183-192.

[171] Takada T, Yoshinari N, Sugiishi S, Kawase H, Yamani T, Noguchi T. *Effect of restraint stress on the progression of experimental periodontitis in rats.* J Periodontol 2004;75:306-315.

[172] Tanaka M, Ejiri S, Toyooka E, Kohno S, Ozawa H. *Effects of ovariectomy on trabecular structures of rat alveolar bone.* J Periodont Res 2002;37:161-165.

[173] Taylor GW, Burt BA, Becker MP, Genco RJ, Shlossman M, Knowler WC, Pettitt DJ. *Non-insulin dependent diabetes mellitus and alveolar bone loss progression over 2 years*. J Periodontol 1998;1:76-83.

[174] Taylor JJ, Preshaw PM, Donaldson PT. *Cytokine gene polymorphism and immunoregulation in periodontal disease.* Periodontol 2000;35:158-182.

[175] Tervonen T, Karjalainen K. *Periodontal disease related to diabetic status. A pilot study of the response to periodontal therapy in type 1 diabetes*. J Clin Periodontol 1997;7:505-510.

[176] Tezal M, Wactawski-Wende J, Grossi SG, Ho AW, Dunford R, Genco RJ. *The relationship between bone mineral density and periodontitis in postmenopausal women.* J Periodontol 2000;71:1492-1498.

[177] Timmerman MF, Van der Weijden GA, Abbas F, Arief EM, Armand S, Winkel EG, Van Winkelhoff AJ, Van der Velden U. *Untreated periodontal disease in Indonesian adolescents. Longitudinal clinical data and prospective clinical and microbiological risk assessment.* J Clin Periodontol 2000;27:932-942.

[178] Tomar SL, Asma S. *Smoking-attributable periodontitis in the United States: findings from NHANES III*. J Periodontol 2000;71:743-751.

[179] Torrungruang K, Nisapakultorn K, Sitdhibhisal S, Tamsailom S, Rojanasomsith K, Vanichjakvong O, Prapakamol S, Premsirinirund T, Pusiri T, Jaratkulangkoon O, Kusump S, Rajatanavin R. *The effect of cigarette smoking on the severity of periodontal disease among older Thai adults*. J Periodontol 2005;76:566-572.

[180] Tran SD, Rudney JD, Sparks BS, Hodges JS. *Persistence presence of Bacteroides forsythus as a risk factor for attachment loss in a population with low prevalence and severity of adult periodontitis.* J Periodontol 2001;72:1-10.

[181] Van der Velden U, Varoufaki A, Hutter JW, Xu L, Timmerman MF, Van Winkelhoff AJ, Loos BG. *Effect of smoking and periodontal treatment on the subgingival microflora.* J Clin Periodontol 2003;30:603-610

[182] Van der Weijden GA, de Slegte C, Timmerman MF, Van der Velden U. *Periodontitis in smokers and non-smokers: intra-oral distribution of pockets.*J Clin Periodontol 2001;28:955-960.

[183] Wactawski-Wende J, Grossi SG, Trevisan M, Genco RJ, Tezal M, Dunford RG, Ho AW, Hausmann E, Hreshchyshyn MM. *The role of osteopenia in oral bone loss and periodontal disease*. J Periodontol 1996 67(Suppl 10):1076-1084.

[184] Wagner J, Kaminski WE, Aslanidis C, Moder D, Hiller K-A, Christgau M, Schmitz G, Schmalz G. *Prevalence of OPG and IL-1 gene polymorphisms in chronic periodontitis*. J Clin Periodntol 2007;34:823-827.

[185] Wendell KJ, Stein SH. *Regulation of cytokine production I human gingival fibroblasts following treatment with nicotine and lipopolysaccharide.* J Periodontol 2001;72:1038-1044.

[186] Westfelt E, Rylander H, Blohme G, Jonasson P, Lindhe J. *The effect of periodontal therapy in diabetics results after 5 years.*J Clin Periodontol 1996;23:92-100.

[187] Weyant RJ, Pearlstein ME, Churak AP, Forrest K, Famili P, Cauley JA. *The association between osteopenia and periodontal attachment loss in older women.* J Periodontol 1999;5:85-95.

[188] Wimmer G, Janda M, Wieselmann-Penkner K, Jakse N, Polansky R, Pertl C. *Coping with stress: its influence on periodontal disease.*J Periodontol 2002;73:1343-1351.

[189] Wimmer G, Köhldorfer G, Mischak I, Lorenzoni M, Kallus KW. *Coping with stress:its influence on periodontal therapy*. J Periodontol 2005;76:90-98.

[190] Yasuda H, Shima N, Nakagawa N, Mochizuki SI, Yano K, Fujise N, Sato Y, Goto M, Yamagushi K, Kuriyama M, Kanno T, Murakami A, Tsuda E, Morinaga T, Higashio K. *Identity of osteoclastogenesis inhibitory factor (OCIF) and osteoprotegerin (OPG): A*

mechanism by which OPG/OCIF inhibits osteoclastogenesis in vitro. Endocrinology 1998;139:1329-1337.

[191] Yoshihara A, Seida Y, Hanada N, Miyazaki H. *A longitudinal study of the relationship between periodontal disease and bone mineral density in community-dwelling older adults*. J Clin Periodontol 2004;31:680-684.

[192] Zambom JJ, Grossi SG, Machtei EE, Ho AW, Dunford R, Genco RJ. *Cigarette smoking increases the risk for subgingival infection with periodontal pathogens.*J Periodontol 1996;67(Suppl. 10):1050-1054.

[193] Zinkernagel RM, Doherty PC. *The discovery of MHC restriction.* Immunol Today 1997;18:14-7.

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Chapter XV

The Role of Antimicrobial Peptides in Periodontal Disease[#]

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Abstract

The oral cavity is a warm, moist environment, in which a number of microorganisms colonize and live in harmony as a community, a so-called biofilm. In this environment, antimicrobial peptides may play a critical role in maintaining normal oral health and controlling innate and acquired immune systems in response to continuous microbial challenges in periodontal disease. Two major families of antimicrobial peptides, found in the oral cavity, are defensin and cathelicidin. Members of the defensin family are cysteine-rich peptides, synthesized by plants, insects, and mammals.These peptides vary in length and in the number of disulfide bonds, and have a beta-sheet structure. In the oral cavity, four alpha-defensinsare synthesized and stored in neutrophil granules, which are converted into active peptides by proteolytic processing, while three humanbeta-defensins (hBDs), hBD-1, hBD-2, and hBD-3, are predominantly produced by oral epithelial cells. The only member of the cathelicidin family found in humans is LL-37, an alpha-helical peptide that contains 37 amino acidsand begins with two leucines at its NH_3-terminus. LL-37 is derived from enzymatic cleavage of a precursor peptide, namely,human cationic antimicrobial peptide-18. Clinically, differential expression of antimicrobial peptides has been reported in specific types of periodontal disease, and their presence has been shown in saliva and gingival crevicular fluid. Current evidence suggests that alpha-defensins, beta-defensins, and LL-37 have distinct, but overlapping, roles in antimicrobial and pro-inflammatory activities. Several studies have shown antimicrobial activities of hBD-2, hBD-3, and LL-37 against several periodontal

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pathogens, suggesting their potential role as antimicrobial agents for periodontal disease. The clinical significance of antimicrobial peptides in periodontal disease has recently been demonstrated in morbus Kostmann syndrome, a severe congenital neutropenia, in which chronic periodontal infection in young patients, resulting from a deficiency of neutrophil-derived antimicrobial peptides, causes early tooth loss. Although researchers initially focused their attention on antimicrobial activities, it is now becoming evident that defensins and LL-37 are multifunctional molecules that mediate various host immune responses, and may thus represent essential molecules of innate immunity in periodontal disease. In this chapter, basic knowledge and the clinical importance of antimicrobial peptides in periodontal disease will be discussed in detail.

Introduction

The warm and moist environment in the oral cavity is a unique niche suitable for a number of microorganisms to colonize, proliferate, and live in harmony as a community, a so-called biofilm. Oral epithelium plays a main role as a physical barrier between the microbial biofilm in the external environment and underlying connective tissue and blood vessels. Naturally, this barrier can be disrupted, since the oral epithelium is the only site in the body normally penetrated by a hard tissue, namely, a tooth. The junction between oral epithelium and the tooth is, therefore, considereda site that is readily susceptible to infection from various microorganisms living in dental plaque. Previously, the role of oral epithelium was viewed as that of an innocent bystander. However, it is now apparent that oral epithelial cells can respond to continuous microbial challenges from the dental plaque by production of cytokines, chemokines, and antimicrobial peptides, which enhance inflammation and immune response in periodontal tissues. Uncontrolled inflammation and immune response from excessive production of these pro-inflammatory molecules is considered one of the etiological factors in the pathogenesis of periodontal disease.

In the oral cavity, antimicrobial peptides may play a critical role in maintaining balance between periodontal health and disease. Therefore, their biological and clinical significances, particularly the ones that are pertinent to periodontal disease, will be emphasized in this chapter. These include the differential expression of antimicrobial peptides in healthy and diseased periodontal tissues and in gingival crevicular fluid (GCF), their antimicrobial effects against a variety of periodontal microorganisms, and their novel functions, related to host immune responses in periodontal disease. Furthermore, some recent studies have demonstrated a connection between the deficiencies in antimicrobial peptide production or function and patients affected with some types of periodontitis, highlighting the clinical importance of these antimicrobial peptides.

Twowell-characterized families of antimicrobial peptides, including defensin and cathelicidin, are present in saliva and GCF, and localized in the oral mucosa (Dale and Fredericks, 2005). These peptides include β-defensins that are expressed in the oral epithelial cells, α-defensins that are secreted from neutrophil granules, and LL-37, the only human antimicrobial peptide in the cathelicidin family, which mainly derives from neutrophil granules and to a lesser extent from oral epithelial cells (Dale et al, 2001). The synthesis of some of these antimicrobial peptides can be considerablyup-regulated upon exposure to oral microorganisms; thus, these peptides are regarded as essential effector molecules in innate immunity. In this chapter, basic knowledge, regarding expression and regulation of defensins

and LL-37, as well as their antimicrobial activities and other functions, will be extensively reviewed. However, a review of other antimicrobial peptides present in the oral cavity, such as calprotectin, adrenomedullin, histatins, etc., is beyond the scope of this chapter and will not be discussed.

General Information on Human Cathelicidin and Defensin

Cathelicidin is a family of antimicrobial peptides that contain a cathelin domain at their NH_3-terminus and an antimicrobial domain at their COOH-terminus (Zanetti et al, 1995). Whereas the amino acid sequence of the cathelin domain is conserved throughout animal species tested to date, the sequence of the antimicrobial domain exhibits considerable variations, accounting for various molecular structures, such as α-helix, β-sheet, etc., possibly reflecting the nature of microbial diversity. The cathelin domain, categorized as a member of the cystatin family (Ritonja et al, 1989), primarily functions as a cathepsin L inhibitor, from which the name of this domain is derived (Kopitar et al, 1989). However, it was later demonstrated that this domain also possesses an antimicrobial function against *Escherichia coli* and methicillin-resistant*Staphylococcus aureus* (Zaiou et al, 2003), yet its antimicrobial mechanism is still largely unknown. The first cathelicidin antimicrobial peptide was isolated from bovine neutrophils (Romeo et al, 1988). Subsequently, several cathelicidin peptides were identified in various mammals, particularly humans. The only cathelicidin in humans, LL-37, is derived from proteolytic processing of a precursor peptide, human cationic antimicrobial protein-18 (hCAP-18), and contains two leucines at its NH_3-terminus (Agerberth et al, 1995; Cowland et al, 1995).

Defensin is a family of small cationic antimicrobial peptides,containing six unique cysteine amino acids that form three disulfide bonds, functioning in stabilization of their β-sheet structure (Zasloff, 2002; Ganz, 2003). Moreover, these peptides, comprising several positively charged amino acids that favorably interact with negatively charged microbial membranes, can form a complex structure, such as a dimeric structure (Hill et al, 1991). In addition, the defensin peptides contain both hydrophobic and hydrophilic domains in their molecules, a so-called amphipathic structure. All of these properties, thus, make the defensins suitable for membrane integration that eventually leads to a pore formation in the membrane. The pore-forming mechanism of the defensins is then believed to be a crucial process in their antimicrobial function. Therefore, it has been shown by a number of studies that the defensins exert their broad spectrum of antimicrobial activities against gram-negative and gram-positive bacteria, fungi, and some enveloped viruses (Ganz, 2003).

The human defensin family can be further divided into two subfamilies, i.e., α-defensin and β-defensin subfamilies. In the α-defensin subfamily, four of the six α-defensins, human neutrophil peptide-1, -2, -3, and -4 (HNP-1, -2, -3, and -4), are synthesized and stored in neutrophil granules (Ganz et al, 1985; Wilde et al, 1989), while the other two α-defensins, human defensin-5 and -6 (HD-5 and -6), are synthesized and stored in the granules of Paneth cells, specialized epithelial cells located at the crypts of Lieberkühn of the small intestine (Jones and Bevins, 1992; 1993). Being encoded by the same gene, the pro-peptide of HNP-1, -2, and -3 comprises 94 amino acids, which is successively cleaved by putative proteolytic

enzymes, yielding different sizes of the mature peptides that are stored in azurophilic granules (Valore and Ganz, 1992). The number of amino acids in the mature peptides of HNP-1 to HNP-3 varies from 29 to 30 amino acids. On the other hand, HD-5 and HD-6 are stored in Paneth cell granules as pro-peptides, and are subsequently activated by trypsin digestion upon release into the intestinal lumen (Ghosh et al, 2002). HNP-4 is encoded by another gene, and its amino acid sequence completely differs from that of HNP-1, HNP-2, and HNP-3, leaving only the identical characteristic cysteines and some arginines (Wilde et al, 1989).

In the β-defensin subfamily, four humanβ-defensins, human β-defensin-1, -2, -3, and -4 (hBD-1, -2, -3, and -4), are principally expressed in epithelial cells that cover several tissues and organs, particularly skin and the mucosal surfaces of gastrointestinal, respiratory, and urogenital tracts, whereas hBD-5 and hBD-6 are expressed only in epididymis (Semple et al, 2003). However, only hBD-1, -2, and -3 are found in the oral cavity (Abiko et al, 2007). HBD-1 and hBD-2 peptides are localized in differentiated epithelial cells within the suprabasal layers of normal gingival epithelium (Dale et al, 2001), whereas hBD-3 peptide is expressed in undifferentiated epithelial cells within the basal layer (Lu et al, 2005), suggesting a potential role for hBD-3 as a mediator to signal the underlying connective tissue cells. HBD-1 is constitutively expressed in several epithelial cell types studied to date, especially gingival epithelial cells (Krisanaprakornkit et al, 1998), whereas expression of hBD-2, hBD-3, and hBD-4 is inducible upon stimulation with pro-inflammatory cytokines or contact with microorganisms. The regulation of human β-defensins will be discussed below.

Expression and Regulation of Human Cathelicidin and Defensins

Human cathelicidin is mainly isolated from neutrophil granules in the amount of 0.627 micrograms per one million neutrophils (Sørensen et al, 1997). After synthesis, human cathelicidin is stored in granules distinct from those that store proteolytic enzymes, such as neutrophil elastase, proteinase-3, etc., to prevent premature activation of the cathelicidin peptide inside the neutrophils. Upon being released into neutrophil phagosomes after bacterial phagocytosis or being released into extracellular environment, the neutrophil cathelicidin is proteolytically cleaved into a mature LL-37 peptide by the proteinase-3 (Sørensen et al, 2001). In addition to regulation of cathelicidin activation by enzymatic cleavage in human neutrophils, cathelicidin expression in other cell types is controlled by exposure to microorganisms, growth factors, and differentiating agents. For instance, LL-37 expression in skin keratinocytes and gastric epithelial cells is induced by *Staphylococcus aureus* and *Helicobacter pyroli*, respectively (Midorikawa et al, 2003; Hase et al, 2003). Furthermore, LL-37 expression in skin keratinocytes is up-regulated by insulin-like growth factor-I and vitamin D, known to promote wound healing and differentiation, respectively (Sørensen et al, 2003; Weber et al, 2005). In addition, LL-37 expression in gastric and small intestinal epithelial cells is induced by short chain fatty acids, including butyrate, via mitogen activated protein (MAP) kinases (Schauber et al, 2003).

Expression of LL-37 can also be found in natural killer cells, monocytes, B- and T-lympocytes (Agerberth et al, 2000), mast cells (Di Nardo et al, 2003), epithelial cells lining respiratory (Bals et al, 1998) and gastrointestinal tracts (Tollin et al, 2003), reproductive

organs (Agerberth et al, 1995; Frohm Nilsson et al, 1999; Malm et al, 2000), salivary glands (Murakami et al, 2002a), sweat glands (Murakami et al, 2002b), and in inflammatory skin disorders (Frohm et al, 1997). In the oral cavity, LL-37 is expressed in buccal and tongue mucosa (Frohm Nilsson et al, 1999), and its expression is up-regulated in the inflamed gingival tissues (Hosokawa et al, 2006). Correspondingly, the concentrations of LL-37 in the gingival tissue, whether derived from neutrophils or from gingival epithelium, correlate positively with the depth of the gingival crevice, suggesting that the LL-37 levels may be used as one diagnostic tool in inflammatory periodontal disorders (Hosokawa et al, 2006). In addition, LL-37 peptide is detected in saliva (Murakami et al, 2002a) and GCF (Puklo et al, 2008), and LL-37 levels in GCF are significantly elevated in patients with chronic periodontitis compared to those in patients with gingivitis or to those in healthy volunteers (Türkoğlu et al, 2009). However, it is likely that LL-37 present in saliva and GCF originates mostly from neutrophil granules (Dale and Fredericks, 2005).

The neutrophil α-defensin gene (*DEFA1*) is located on chromosome 8 (8p23) (Sparkes et al, 1989), and the number of suchgenesin different individuals varies from two to three genes per diploid cell (Mars et al, 1995). HNP-1, HNP-2, and HNP-3 mRNAs are mainly expressed in neutrophils, and their respective proteins were first characterized from azurophilic granules (Ganz et al, 1985) that also comprise other antimicrobial peptides, such as myeloperoxidase, cathelicidin, etc. Moreover, expression of HNP-1, HNP-2, and HNP-3 can be detected in lymphocytes (Blomqvist et al, 1999; Agerberth et al, 2000) and Langerhans cells in the vicinity of epithelial dysplasia adjacent to precancerous lesions and oral squamous cell carcinoma (Mizukawa et al, 1999), but their expression is not found in normal oral mucosa. They are also present in ductal cells of submandibular salivary glands from patients with oral cancer (Mizukawa et al, 2000).

With respect to periodontal tissue, the detectable amounts of HNP-1, HNP-2, and HNP-3 in GCF can vary from 270 to 2000 nanogram per site (or approximately equivalent to mg/ml) (McKay et al, 1999), which is sufficient for their antimicrobial function in periodontium. By virtue of matrix assisted laser desorption ionization mass spectrometry (MALDI-MS), it has been demonstrated that HNP-1 is most abundant in GCF, whereas HNP-3 is least abundant (Lundy et al, 2005). Moreover, the concentrations of HNP-1 to HNP-3, as well as those of LL-37 and hBD-3, have been quantified in saliva. These concentrations (up to twelve μg/ml) are variable in the human population (Tao et al, 2005). In addition, the median levels of HNP-1 to HNP-3 in saliva are significantly higher in children without dental caries than in those with dental caries experience, whereas the median levels of LL-37 and hBD-3 do not correlate with caries experience (Tao et al, 2005), suggesting the protective role of neutrophilα-defensins against dental caries.

Enteric α-defensin genes are located on chromosome 8 in the same vicinity as *DEFA1*, suggesting the duplication of α-defensin genes during evolution (Bevins et al, 1996). Up to now, only very weak HD-5 expression has been identified in a few oral tissue samples, whereas HD-6 expression is not detectable at all, indicating that enteric α-defensins do not play any role in the innate immunity of the oral cavity (Dunsche et al, 2001).

β-defensins are somewhat larger than α-defensins. Although 28 β-defensin genes have been discovered by computer searching of the human genome (Schutte et al, 2002), expression of only six human β-defensins, hBD-1 to hBD-6, has been characterized to date in human tissues and organs. HBD-1 is the first human β-defensin, isolated from hemofiltrate

passing through the kidney at the nanomolar levels (Bensch et al, 1995). The gene encoding hBD-1, *DEFB1*, is on chromosome 8, in close proximity to *DEFA1*, around 100-150 kilobases apart (Liu et al, 1997). However, both the amino acid sequence and the pairing between two cysteine amino acids that form the disulfide bond in hBD-1 greatly differ from both the sequence and pairing in HNP-1; thus, creating a new β-defensin subfamily. *DEFB1* contains two exons with one large 6962 base pair (bp) intron (Liu et al, 1997). The two exons encode a 362 bp complementary DNA (cDNA) that is translated into an hBD-1 pro-peptide (Liu et al, 1997). The hBD-1 pro-peptide is subsequently cleaved to yield several hBD-1 mature peptides, ranging from 36 to 47 amino acids long. Widespread and low expression of hBD-1 has been detected in various epithelia lining several organs, including trachea, bronchus, prostate gland, mammary gland, placenta, thymus, testis, skin, small intestine (Zhao et al, 1996), pancreas and kidney – especially, the collecting duct, distal tubule, and loop of Henle – (Schnapp et al, 1998), vagina, endometrium, Fallopian tube (Valore et al, 1998), and salivary glands (Bonass et al, 1999; Sahasrabudhe et al, 2000).

In the oral mucosa, hBD-1 expression is found in gingival epithelium, but is not associated with the amount of IL-8 expression in the gingival tissue (Krisanaprakornkit et al, 1998). In other words, the amount of hBD-1 expression in gingival tissue does not correlate with the degree of tissue inflammation, but varies among different individuals (Krisanaprakornkit et al, 1998). Moreover, confluent cultured gingival epithelial cells constitutively express hBD-1 mRNA at baseline levels; however, its expression is up-regulated in a post-confluent culture, representing the state of cellular differentiation*in vitro* (Dale et al, 2001). In this study, the state of differentiation is shown by increased mRNA expression of profilaggrin, a late marker for differentiation. Consistent with the increased hBD-1 mRNA expression in the post-confluent culture, hBD-1 mRNA and peptide are localized in the suprabasal layers of oral epithelium *in vivo* (Dale et al, 2001). On the other hand, it has been demonstrated that increased hBD-1 expression can, in turn, induce differentiation in skin keratinocytes (Frye et al, 2001).

By using a protein chip array together with surface enhanced laser desorption/ionization (SELDI) andtime-of-flight mass spectrometry, hBD-1 peptide at a molecular mass of about 4.7 kilodalton (kDa) is detected in culture medium of gingival epithelial cells (Diamond et al, 2001). Unlike known concentrations of neutrophilα-defensins in GCF, the precise levels of hBD-1 present in GCF have not yet been accurately quantified. Highly variable amounts of hBD-1 peptide have been found in saliva and GCF, collected from different normal individuals (Diamond et al, 2001). It is possible that salivary ductal cells may also contribute some hBD-1 peptide detected in saliva in addition to hBD-1 peptide synthesis by oral epithelial cells (Sahasrabudhe et al, 2000). It is noteworthy that hBD-1 and hBD-2 are neither expressed in cultured gingival fibroblasts (Krisanaprakornkit et al, 1998; 2000) nor found in the underlying connective tissue of the oral mucosa (Dale et al, 2001).

The second humanβ-defensin, hBD-2, was first isolated in large amounts from psoriatic skin keratinocytes (Harder et al, 1997a). The gene encoding hBD-2 is *DEFB4*, which is located on chromosome 8, region 8p22-p23.1, in close proximity to *DEFA1* and *DEFB1* (Harder et al, 1997b). *DEFB4* contains one 1639 bp intron (Liu et al, 1998), and two small exons that encode a signal peptide domain and a mature peptide, whose sizes are 23 and 41 amino acids long, respectively (Harder et al, 1997b). Expression of both hBD-1 and hBD-2 is localized in the suprabasal layers of normal epidermis (Ali et al, 2001), identical with their

expression in normal oral mucosa (Dale et al, 2001). HBD-2 peptide is stored in lamellar granules in the spinous layer of epidermis, and later released into the extracellular environment with other lipids in the granular layer, suggesting that lipids covering the skin function as a natural barrier against water permeability and microbial invasion due to the presence of antimicrobial peptides (Oren et al, 2003).

As with the inducible expression of hBD-2 by microorganisms and pro-inflammatory cytokines in other cell types, hBD-2 mRNA is up-regulated in cultured gingival epithelial cells in response to stimulation with IL-1β, TNF-α, phorbol ester, a potent epithelial activator, and Gram-negative periodontal bacteria, including *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, and *Porphyromonas gingivalis* (Mathews et al, 1999; Krisanaprakornkit et al, 2000; Noguchi et al, 2003; Chung et al, 2004; Taguchi and Imai, 2006; Laube et al, 2008). Nevertheless, unlike the critical role of CD14, a lipopolysaccharide (LPS) co-receptor, and nuclear factor-kappa B (NF-κB) in hBD-2 induction in respiratory epithelial cells and mononuclear phagocytes (Becker et al, 2000; Harder et al, 2000; Tsutsumi-Ishii and Nagaoka, 2002), CD14 and NF-κB are neither critical nor essential for hBD-2 up-regulation in gingival epithelial cells (Krisanaprakornkit et al, 2002). In fact, a purified LPS fraction of either *Fusobacterium nucleatum* or *Aggregatibacter actinomycetemcomitans* is a poor hBD-2 activator in gingival epithelial cells (Krisanaprakornkit et al, 2000; Laube et al, 2008, respectively). Furthermore, p38MAP kinase and c-Jun N-terminalMAP kinase (JNK) control hBD-2 mRNA up-regulation in response to *Fusobacterium nucleatum* in gingival epithelial cells (Krisanaprakornkit et al, 2002). Likewise, the MAP kinase pathways, but not the NF-κB transcription factor, are critical for hBD-2 up-regulation by the outer membrane protein 100 (Omp100; named after its molecular mass) of *Aggregatibacter actinomycetemcomitans* (Ouhara et al, 2006). Taken together, these findings suggest different cellular receptors and intracellular signaling mechanisms to control hBD-2 up-regulation by different stimulants in distinct cell types.

In addition to the involvement of p38MAP kinase and JNK in hBD-2 up-regulation by *Fusobacterium nucleatum*, it is shown that an increase in intracellular calcium ion and phosphorylated phospholipase D, two important molecules in regulating epithelial cell differentiation (Exton, 1999; Bollag et al, 2005), are involved in hBD-2 up-regulation by *Fusobacterium nucleatum* (Krisanaprakornkit et al, 2003; 2008). It is noteworthy that treatment of gingival epithelial cells with either exogenously added calcium ions or thapsigargin, an inhibitor of the sarcoendoplasmic reticulum calcium (SERCA) pump, an inhibitor that leads to continuous calcium ion release from its intracellular storage, induces hBD-2 mRNA, whereas BAPTA-AM, a cell permeable calcium chelator, blocks hBD-2 mRNA up-regulation by *Fusobacterium nucleatum* and thapsigargin in a dose-dependent manner (Krisanaprakornkit et al, 2003). In summary, the regulation of hBD-2 expression can be controlled by both inflammation from bacteria and epithelial differentiation.

Consistent with this conclusion, the strongest hBD-2 expression in gingival tissue is found at the gingival margin, adjacent to microbial plaque accumulation, and hBD-2 expression is localized in differentiated epithelial cells within the suprabasal layers of gingival epithelium (Dale et al, 2001). Moreover, the localization of hBD-2 peptide is found not only in cultured gingival epithelial cells that express involucrin, another marker for differentiation, but also in stimulated cells with infectious and pro-inflammatory stimulants (Dale et al, 2001). In contrast, neither hBD-1 nor hBD-2 is expressed in junctional epithelium

(Dale et al, 2001), which consists of relatively undifferentiated epithelial cells, implying that the junctional epithelium may be more susceptible to infection than other areas of gingival epithelium because of the lack of some antimicrobial peptides. However, it is probable that other antimicrobial peptides, such as α-defensins, LL-37, etc., released from neutrophils that transmigrate from blood vessels into the junctional epithelium and gingival crevice, may perform this antimicrobial function instead (Dale and Fredericks, 2005).

Using biochemical and molecular biology techniques, the gene encoding hBD-3 (*DEFB103*) has been cloned from human skin keratinocytes and alveolar epithelial cells, and the amino acid composition of hBD-3 has been sequenced and classified as a novel peptide in the β-defensin subfamily (Harder et al, 2001). *DEFB103*,containing two small exons, is located 13 kb upstream from *DEFB4* that encodes hBD-2 on chromosome 8 (Jia et al, 2001). HBD-3 cDNA is translated into an hBD-3 pro-peptide that comprises a signal peptide domain (22 amino acids long) and a mature peptide (45 amino acids long). The amino acid sequence of hBD-3 is 43% identical to that of hBD-2 (Jia et al, 2001).

In addition to skin keratinocytes, hBD-3 is expressed in various epithelia lining several tissues, including gingiva (Jia et al, 2001; Dunsche et al, 2002), tonsils (Harder et al, 2001), esophagus, trachea, placenta, and fetal thymus glands (Jia et al, 2001). In the oral cavity, hBD-3 mRNA and peptide are localized in the basal layer of normal gingival epithelium (Lu et al, 2005), whereas hBD-1 and hBD-2 are expressed in the suprabasal layers (Dale et al, 2001). Furthermore, hBD-3 mRNA is expressed in both inflamed and non-inflamed epithelium and salivary glands (Dunsche et al, 2001), and its expression is up-regulated in leukoplakia and oral lichen planus (Nishimura et al, 2003). *In vitro*, hBD-3 mRNA expression is induced in cultured epithelial cells that are stimulated with IFN-γ, TNF-α, and IL-1β (García et al, 2001; Harder et al, 2001; Jia et al, 2001), although IFN-γ does not up-regulate hBD-2 mRNA (García et al, 2001). Consistent with the findings obtained from these studies, it was later demonstrated that IFN-γ is a primary inducer for hBD-3 expression, whereas IL-1β and TNF-α are major stimulants for hBD-2 expression (Joly et al, 2005).

With respect to up-regulation of hBD-3 by oral microorganisms, hBD-3 mRNA expression is induced by live nonperiodontopathic bacteria (Ji et al, 2007a), including *Streptococcus sanguinis* and *Streptococcus gordonii*, and some periodontopathic bacteria, including *Aggregatibacter actinomycetemcomitans* (Feucht et al, 2003), *Prevotella intermedia*, and *Fusobacterium nucleatum* (Ji et al, 2007a). In contrast, three well known causative pathogens in chronic periodontitis, including *Porphyromonas gingivalis*, *Tanerella forsythia*, and *Treponema denticola*, down-regulate hBD-3 mRNA expression, as well as IL-8 production and secretion in an oral epithelial cell line (Ji et al, 2007a). This indicates that these so-called "red-complex" periodontal pathogens may suppress innate immune responses of oral epithelial cells by an immune-evading mechanism, known as "chemokine paralysis" (Darveau et al, 1998). Furthermore, the red-complex bacteria can tolerate the host immune response by being more resistant to LL-37 and phagocytosis by neutrophils (Ji et al, 2007b), indicating their strong implication with chronic periodontal infection.

Antimicrobial Activity

Up to the present, there have been an enormous number of *in vitro* studies, showing the antimicrobial activity of LL-37 and human defensins against various pathogens associated with a variety of human diseases. All of these studies cannot be completely mentioned in this chapter due to the space limitation. Therefore, the scope of this topic will be restricted to the antimicrobial effects on oral pathogens, especially the ones associated with periodontal disease. In the oral cavity, the warm temperature and moistened mucosal and tooth surfaces are suitable for microbial colonization and then the formation of biofilm, so-called dental plaque. The dental plaque is essential for some specific oral microorganisms to survive and thrive in this complex community. It is conceivable that the exopolysaccharide-producing plaque can protect oral microorganisms from exposure to antibiotics, or antimicrobial peptides in the context of this discussion.As with antibiotics, it is, therefore, likely that plaque microorganisms are more resistant to destructionby antimicrobial peptides than are planktonic microrganisms present in the saliva. Consequently, antimicrobial peptides can be regarded as one of the selective pressures that oral microorganisms must overcome in order to establish colonies in the dental plaque.

Furthermore, it should be emphasized that the results obtained from most studies that examine the susceptibility of one or more microbial species to individual antimicrobial peptides*in vitro* may not represent the real effectiveness of antimicrobial peptides due to the complexity of interactions between host and microorganisms or between two different types of microorganisms in the dental plaque. However, it is rather difficult to evaluate the effectiveness of antimicrobial peptides in such a complicated situation *in vivo*. Fortunately, some recent *in vivo* studies have shed light into the clinical significance of antimicrobial peptides for periodontal homeostasis. In this regard, it has been shown that genetic and acquired deficiencies of some antimicrobial peptides are associated with the pathogenesis of some types of periodontitis (Pütsep et al, 2002; Puklo et al, 2008), and this will be discussed under the next heading.

Other factors that influence the antimicrobial effects of some antimicrobial peptides are high salt concentrations that are shown to inhibit antimicrobial functions in other parts of the body (Goldman et al, 1997; Midorikawa et al, 2003) and the presence of inhibitors in serum (Tanaka et al, 2000). However, in the oral cavity, antimicrobial peptides may not be affected by these factors, since the peptides function at the mucosal surface, where the concentrations of salt or inhibitors, diluted with saliva, are too low to exert any significant inhibitory action.

At the outsetof the study of the antimicrobial effects on oral bacteria, the bactericidal activity of LL-37 was tested against different strains of *Aggregatibacter actinomycetemcomitans* and *Capnocytophaga* spp., which are implicated in the pathogenesis of juvenile periodontitis and gingivitis, respectively (Tanaka et al, 2000). It was found that the concentrations of LL-37 (below 12 μg/ml) already killed all strains of these two bacteria by 99%. Subsequently, under a more detailed investigation into the antimicrobial effects of LL-37 against different kinds of periodontal bacteria, involved with various stages of dental plaque formation, it was demonstrated that the early colonizing yellow-complex bacteria, such as oral *Streptococci*, *Actinomyces*, etc., and the bridging orange-complex bacterium, i.e.,*Fusobacterium nucleatum*, are susceptible to the bactericidal activity of LL-37 with low minimum inhibitory concentrations (MICs) in μg/ml (Ji et al, 2007b). Similar results have

also been obtained from another study (Ouhara et al, 2005), which shows the antimicrobial effects of LL-37 against various gram-positive oral *Streptococci*. In contrast, the red-complex periodontopathic bacteria, including *Porphyromonas gingivalis*, *Tannerella forsythensis*, and *Treponema denticola*, are more resistant to LL-37 than are other bacteria (Ji et al, 2007b), suggesting their strong involvement with periodontitis.

Furthermore, LL-37 exerts its candidacidal activity by disrupting the yeast cell membrane, leading to membrane fragmentation and a release of intracellular contents, such as adenosine triphosphate (den Hertog et al, 2005). With respect to the antimicrobial activity of neutrophilα-defensins, oral microorganisms are usually resistant to HNP-1 to HNP-3, even though a synergistic antimicrobial effect is revealed between HNP-1 and LL-37 against *Escherichia coli* and *Staphylococcus aureus* (Nagaoka et al, 2000).

The antimicrobial activities of hBD-1, hBD-2, and hBD-3 peptides have been tested against different strains of gram-negative and gram-positive oral bacteria and fungi in several *invitro* studies. In brief, it is found that, among thesethree humanβ-defensins, hBD-3 has the strongest antibacterial activity against oral *Streptococci* and some periodontal bacteria, especially all strains of *Fusobacterium nucleatum*, while hBD-1 and hBD-2 are less effective against both oral gram-positive and gram-negative bacteria (Ouhara et al, 2005). This may be owing to the strong basic property of hBD-3 due to several positively charged amino acids in its molecule (Schibli et al, 2002). However, hBD-2 exerts its antimicrobial activity well with cariogenic bacteria, including *Streptococcus mutans* and *Streptococcus sobrinus* (Nishimura et al, 2004). Generally, aerobic bacteria are more susceptible to hBD-2 and hBD-3 peptides than are anaerobic bacteria (Joly et al, 2004).Although the antimicrobial activity of β-defensins is normally inhibited by high salt concentrations, as shown in other studies (Goldman et al, 1997; Midorikawa et al, 2003), the antimicrobial activity of hBD-3 against periodontal and cariogenic bacteria is not much influenced by high salt concentrations (Ouhara et al, 2005). It can be concluded that, among the antimicrobial peptides of the defensin and cathelicidin families, hBD-3 and LL-37 exhibit the greatest degrees of antimicrobial effects against various oral bacteria, especially most aerobic bacteria and some periodontal bacteria. Although the red-complex periodontopathic bacteria are more resistant to hBD-3 and LL-37, it is likely that hBD-3 and LL-37 may still play a role in the pathogenesis of periodontal disease by reducing the number of early colonizing and bridging bacteria so that the late colonizers, including the red-complex periodontopathic bacteria, cannot colonize and thrive in dental plaque.

Interestingly, some pathogenic bacteria have evolved other virulence mechanisms that enable them to resist the activity of antimicrobial peptides. For example, antimicrobial peptides can be degraded by distinct enzymes secreted from bacterial pathogens, including SufA, a novel subtilisin-like serine protease of *Finegoldia magna* (Karlsson et al, 2007), streptopain of *Streptococcus pyogens*, elastase of *Pseudomonas aeruginosa*, gelatinase of *Enterococcus faecalis* (Schmidtchen et al, 2002), and the 50 kDa metalloprotease (ZapA) of *Proteus mirabilis* (Belas et al, 2004). By analogy, *Porphyromonas gingivalis*, one of the red-complex bacterial triad, can also be resistant to the bactericidal activity of antimicrobial peptides due to its ability to synthesize a group of enzymes, called gingipains. In fact, it has been recently demonstrated that the gingipains efficiently degrade several different antimicrobial peptides, including HNP-1, hBD-1, hBD-2, and hBD-3 (Carlisle et al, 2009). However, it was formerly shown that the degradation of antimicrobial peptides by gingipains

does not appear to contribute to the resistance of *Porphyromonas gingivalis* to the antimicrobial action (Bachrach et al, 2008).

The possible alternative mechanisms for the resistance of *Porphyromonas gingivalis* may be due to the possibility that gingipains secreted from *Porphyromonas gingivalis* may prevent destruction of its commensal bacteria, i.e., *Fusobacterium nucleatum*, which is easily destroyed by antimicrobial peptides. Otherwise, gingipains and proteases released from *Porphyromonas gingivalis* and *Prevotella intermedia*, respectively, may inactivate cystatins, inhibitors that function against endogenously-derived proteases, such as host cathepsins, etc. This ultimately releases cathepsins from their tight control by cystatins. The active cathepsins, including cathepsin B, L, and S in the cysteine protease family, may then proteolytically degrade antimicrobial peptides, resulting in depletion of antimicrobial activity (Taggart et al, 2003). The gingipains and other virulence factors make *Porphyromonas gingivalis* one of the critical periodontal pathogens, and antimicrobial peptides may then be regarded as an important determinant for the "normal" and "diseased" states of periodontium.

As with *Porphyromonas gingivalis*, *Treponema denticola*, another red-complex periodontal pathogen, is resistant to the antimicrobial activity of humanβ-defensins, but by other distinct mechanisms, since *Treponema denticola* does not produce degrading enzymes. These mechanisms include an efflux pump of defensin peptides that enter the cytoplasm (Brissette and Lukehart, 2007) and reduction of defensin binding to the microbial surface due to the lack of LPS (Brissette and Lukehart, 2002). Furthermore, *Treponema denticola* cannot induce the host innate immune response, i.e., expression of hBD-2 and IL-8, in gingival epithelial cells (Brissette et al, 2008). The immune tolerant mechanisms of *Treponema denticola*, including resistance to the antimicrobial effect of antimicrobial peptides and silencing host innate immunity, may, therefore, partly explain the strong association of *Treponema denticola* with chronic periodontitis.

Immunoregulatory Effects

In addition to its antimicrobial activities, LL-37 can elicit host innate and acquired immune responses. For example, LL-37 inhibits the binding of endotoxin LPS to its receptor complex, comprising Toll-like receptors (TLRs) and CD14, which results in prevention of sepsis (Fukumoto et al, 2005; Mookherjee et al, 2006) and suppression of the synthesis of nitric oxide (Ciornei et al, 2003), TNF-α, prostaglandin E_2 (PGE_2), monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein-2 (MIP-2) (Ohgami et al, 2003). Moreover, LL-37 can block macrophage stimulation with lipoteichoic acid and lipoarabinomannan, indicating that LL-37 can bind to various molecules on bacterial cell membranes (Scott et al, 2002).

LL-37 chemoattracts monocytes, neutrophils, CD4 T lymphocytes, and eosinophils along its concentration gradient via a G-protein coupled receptor, namely, formyl peptide receptor-like 1 (FPRL1), on these cells (De Yang et al, 2000; Tjabringa et al, 2006). However, the appropriate LL-37 concentrations fall within the range between 10^{-7} and 10^{-5} molar, which are far greater than those of chemokines used in chemotaxis. In this regard, it is possible that LL-37 can play a role as a chemoattractant at inflamed periodontal sites only when elevated concentrations of LL-37 derived from inflamed gingival epithelial cells and granules of

neutrophils, which are abundant in diseased tissues, are sufficient to exert the chemotactic effect. Moreover, LL-37 attracts migration of mast cells in rats (Niyonsaba et al, 2002a) and induces histamine release from mast cell granules via intracellular calcium mobilization (Niyonsaba et al, 2001), leading to enhanced phagocytosis of opsonized microorganisms. LL-37 can also induce dendritic cell differentiation, which then activates cell-mediated acquired immunity through a Th1 profile (Davidson et al, 2004).

Several studies have also shown the inducible effect of LL-37 on the expression of several immune-related genes. For instance, LL-37 can induce expression of chemokines and chemokine receptors (Scott et al, 2002) via MAP kinase pathways (Bowdish et al, 2004). It can also induce expression of intercellular adhesion molecule-1 (Edfeldt et al, 2006), implying an indirect role for LL-37 in chemotaxis in addition to its direct role, as indicated above.LL-37 transactivates an epidermal growth factor receptor (EGFR) through induction of matrix metalloproteinase activity, resulting in interleukin-8 (IL-8) up-regulation and increased cell proliferation in human bronchial epithelial cells (Tjabringa et al, 2003). Similarly, LL-37 enhances IL-8 expression and release by human airway smooth muscle cells, albeit through purinergic receptors (Zuyderduyn et al, 2006). In addition, in the presence of IL-1β, lower LL-37 concentrations can synergistically induce IL-8 synthesis in both human keratinocytes and bronchial epithelial cells (Filewod et al, 2009), and up-regulate expression of IL-6, IL-10, MCP-1, and MCP-3 in peripheral blood mononuclear cells (Yu et al, 2007).

With respect to periodontal cells, we have recently found similar IL-8 mRNA up-regulation by LL-37 in both gingival epithelial cells and gingival fibroblasts in dose- and time-dependent manners (Figure 1). Interestingly, the kinetics of IL-8 up-regulation between these two cell types shows distinct profiles, indicating different signaling pathways controlling IL-8 expression (Figure 1). Therefore, it is possible that LL-37 may be responsible for controlling neutrophil transmigration from blood vessels into diseased periodontal tissue in chronic periodontitis.

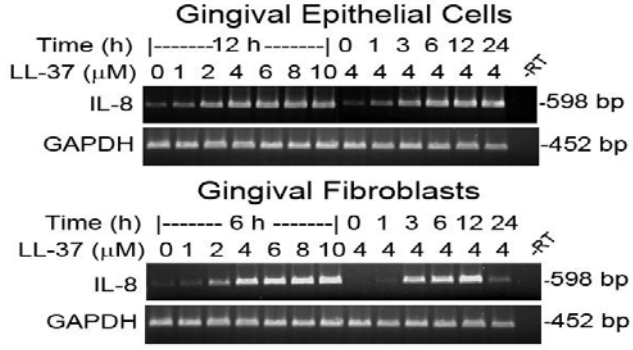

Figure 1. Up-regulation of IL-8 mRNA expression by treatment with various doses (0-10 μM) of LL-37 for indicated times (0-24 hours) in gingival epithelial cells and gingival fibroblasts. Note a dose-dependent increase in IL-8 expression. While IL-8 mRNA was transiently induced by LL-37 in gingival fibroblasts, up-regulation of IL-8 mRNA in gingival epithelial cells accumulated from 0 to 24 hours. Expression of glyceraldehyde phosphate dehydrogenase(GAPDH) was equivalent among all samples, indicating the equal mRNA loadings in this experiment. –RT represents a negative control sample where a reverse transcriptase enzyme was omitted from the reaction.

As with LL-37, neutrophilα-defensins also exert their immunomodulating effects on various types of immune cells. For example, HNP-1 and HNP-2 can induce chemotaxis of T-lymphocytes (Chertov et al, 1996), dendritic cells (Yang et al, 2000), macrophages, and mast cells (Grigat et al, 2007). Neutrophil α-defensins enhance cytokine expression in T-lymphocytes and immunoglobulin G production in B-lymphocytes (Tani et al, 2000), induce IL-8 expression in lung epithelial cells (van Wetering et al, 1997), and promote IL-1β release through posttranslational processing (Perregaux et al, 2002).

With regard to the immunoregulatory effects of humanβ-defensins, hBD-1 and hBD-2 chemoattract immature dendritic cells and memory T-lymphocytes through a G-protein coupled chemokine receptor, i.e., CCR6, indicating the ability of these two β-defensins to bridge innate and acquired immunity (Yang et al, 1999). HBD-1 activates monocyte-derived dendritic cells and promotes the synthesis of several cytokines (Presicce et al, 2009). Moreover, hBD-1 up-regulates expression of CD91, a scavenger receptor that recognizes defensins, on the dendritic cell surface, indicating a positive feedback of dendritic cell activation (Presicce et al, 2009). However, hBD-2, but not hBD-1, enhances chemotaxis of mast cells (Niyonsaba et al, 2002b) and neutrophils treated with TNF-α (Niyonsaba et al, 2004), possibly via a CCR6 that mediates the signal through activation of phospholipase C. Furthermore, hBD-2 induces histamine release from mast cells and prostaglandin D synthesis (Niyonsaba et al, 2001). As with the dissociation of antimicrobial activities from the host immunostimulatory activities of LL-37 (Braff et al, 2005), it has recently been demonstrated that the chemoattractant and antimicrobial activities of β-defensins are exerted by distinct domains, and both of these activities do not rely on the intramolecular disulfide bridges of β-defensins (Taylor et al, 2008).

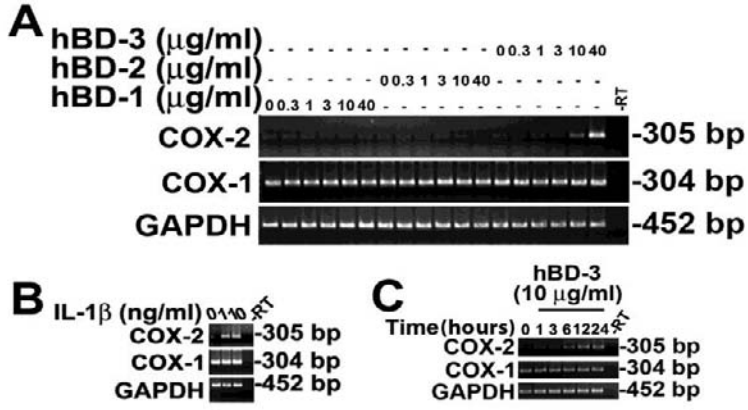

Figure 2.COX-2 mRNA up-regulation by hBD-3. Human gingival fibroblasts were treated for 18 hours with (A) various doses (0-40 μg/ml) of hBD-1, hBD-2, hBD-3, (B) IL-1β as a positive control, (C) 10 μg/ml of hBD-3 for various times (0-24 hours), or left untreated as a negative control. Total RNA was harvested and RT-PCR was conducted to analyze mRNA expression for cyclooxygenase-1 (COX-1), COX-2, and GAPDH. Note constitutive COX-1 mRNA expression, while COX-2 mRNA was up-regulated by hBD-3 treatment in dose- and time-dependent manners. This figure is reproduced from Chotjumlong and co-workers, 2010, with permission from the publisher, Wiley-Blackwell.

Regarding a potential role for humanβ-defensins in modulating host immune responses in periodontal disease, we have very recently shown that only hBD-3, but not hBD-1 or hBD-2, induces mRNA and protein expression of cyclooxygenase-2 (COX-2) in gingival fibroblasts in dose- and time-dependent fashions (Figures 2 and 3, respectively).

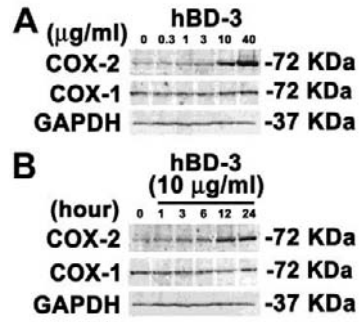

Figure 3. Up-regulation of COX-2 protein by hBD-3 in human gingival fibroblasts. Consistent with COX-2 mRNA up-regulation, COX-2 protein expression was up-regulated by hBD-3 treatment in (A) dose- and (B) time-dependent manners. Note constitutive COX-1 protein expression. This figure is reproduced from Chotjumlong and co-workers, 2010, with permission from the publisher, Wiley-Blackwell.

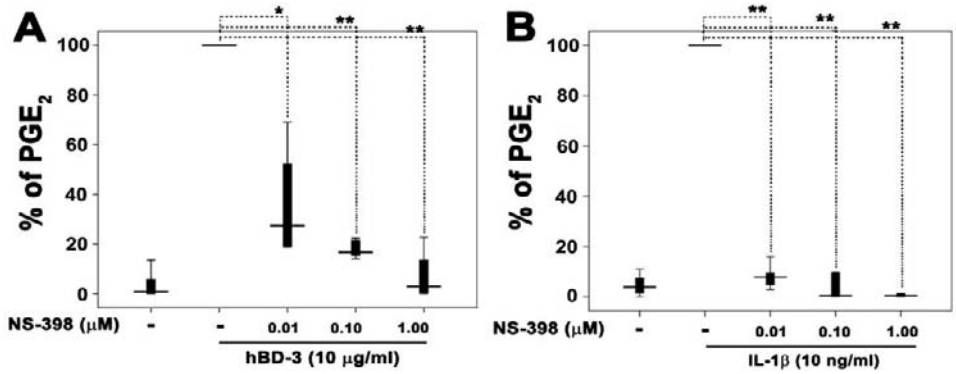

Figure 4. Elevated PGE_2 levels result from induced COX-2 expression. Human gingival fibroblasts were pretreated with indicated doses of NS-398, a specific COX-2 inhibitor, for 30 minutes prior to treatment with either (A) 10 μg/ml of hBD-3 or (B) 10 ng/ml of IL-1β for 18 hours. Cell-free culture supernatants were collected and analyzed for the PGE_2 levels by ELISA. Note a significant inhibition of elevated PGE_2 levels by NS-398 (*, $P < 0.05$; **, $P < 0.01$). This figure is reproduced from Chotjumlong and co-workers, 2010, with permission from the publisher, Wiley-Blackwell.

In comparison to up-regulation of COX-2 mRNAby 1-10 ng/ml of IL-1β, up-regulation by hBD-3 requires much higher concentrations (Figure 2), suggesting that epithelial-derived hBD-3 may act as a local immunomodulator on fibroblasts in adjacent connective tissue, where its concentration is sufficient to reach the low range of μg/ml. This concentration can probably be achieved by persistent inflammation in chronic periodontitis. Furthermore, up-

regulated COX-2 expression by hBD-3 results in raised PGE_2 levels in cell-free culture supernatants (Table 1), which is confirmed by an experiment using a specific inhibitor of COX-2 activity, i.e., NS-398 (Figure 4). In summary, all of these findings suggest the potential role and ability of hBD-3 in initiating localized inflammation within periodontal tissues.

Table 1.HBD-3 treatment results in elevated PGE_2 levels in cell-free culture supernatants in a dose-dependent fashion. The cell-free culture supernatants from Figure 2 were collected and analyzed for PGE_2 concentrations (pg/ml) by ELISA. This table is modified from Chotjumlong and co-workers, 2010, with permission from the publisher, Wiley-Blackwell.

Concentration (μg/ml)	Median PGE_2 concentration (range)
Control	36.40 (34.49-38.32)
hBD-1 0.3	35.91 (33.74-38.08)
hBD-1 1.0	39.22 (36.28-42.16)
hBD-1 3.0	37.42 (34.99-39.85)
hBD-1 10.0	37.35 (34.96-39.73)
hBD-1 40.0	39.61 (35.43-43.78)
hBD-2 0.3	38.30 (35.70-40.89)
hBD-2 1.0	37.58 (35.00-40.16)
hBD-2 3.0	39.64 (34.86-44.42)
hBD-2 10.0	40.51 (38.37-42.66)
hBD-2 40.0	36.82 (32.94-40.69)
hBD-3 0.3	21.33 (20.00-23.00)
hBD-3 1.0	35.55 (22.64-48.31)
hBD-3 3.0	53.16 (48.31-58.00)
hBD-3 10.0	260.59*(260.56-266.03)
hBD-3 40.0	1934.00*(1824.20-2048.10)

*denotes statistically significant difference from untreated control cells at$P<0.05$.

Other Biological Activities

Besides the immunomodulation, LL-37 plays a role in tissue repair by stimulating airway epithelial cell proliferation and wound closure (Shaykhiev et al, 2005) and by activating keratinocyte proliferation and migration in the process of re-epithelialization (Heilborn et al, 2003) via transactivation of EGFR and phosphorylation of the signal transducers and activator of transcription 3 (STAT3) (Tokumaru et al, 2005). Consistent with these *in vitro* studies, the levels of LL-37 decrease in chronic ulcer epithelium (Heilborn et al, 2003), whereas adenoviral transfer of LL-37 to the wound in mice results in a significant improvement of wound healing by enhanced re-epithelialization and granulation tissue formation (Carretero et al, 2008). Furthermore, it has recently been shown that LL-37 can suppress keratinocyte

apoptosis via a COX-2-dependent mechanism (Chamorro et al, 2009), which is in agreement with the function of LL-37 in promoting cell proliferation and tissue repair, as indicated above. Therefore, it is interesting to determine whether LL-37 plays any role in tissue repair and/or regeneration after periodontal surgery.

Interestingly, exogenously added LL-37 into the wound induces angiogenesis that corresponds to an *in vitro* study (Koczulla et al, 2003), which demonstrates endothelial cell proliferation and increased numbers of new blood vessel formation through FPRL1 on cultured endothelial cell membrane in response to LL-37 treatment. As in keratinocyte migration, LL-37 also induces migration of human corneal epithelial cells, as well as expression of IL-1β, IL-6, IL-8, and TNF-α (Huang et al, 2006). Moreover, it has been shown that LL-37 can internalize into human lung epithelial cells through endocytosis, and subsequently accumulates in the perinuclear region (Lau et al, 2005).

There are a number of reports that show other biological effects of neutrophilα-defensins and humanβ-defensins on various cell types. For instance, α-defensins enhance mitosis in some cell types (Murphy et al, 1993), promote tissue repair in airway epithelial cells via MAP kinase pathways (Aarbiou et al, 2002), regulate expression for adhesion molecules on endothelial cells (Chaly et al, 2000), control smooth muscle cell contraction via an α2-macroglobulin receptor (Nassar et al, 2002), induce proliferation of lung fibroblasts and collagen synthesis (Han et al, 2009), and induce expression of some mucin genes, i.e., *MUC5B* and *MUC5AC* (Aarbiou et al, 2004). As with the induction of mucin genes by neutropil α-defensins, it has lately been demonstrated that LL-37 also up-regulates *MUC2* and *MUC3* expression in intestinal epithelial cell lines (Otte et al, 2009). Furthermore, α-defensins affect histamine release from mast cell granules through a G-protein coupled receptor, suggesting their indirect role in vasodilatation (Befus et al, 1999).

Among humanβ-defensins, hBD-2 activates the differentiation of dental pulp mesenchymal cells into odontoblast-like cells, confirmed by up-regulation of dentin sialophosphoprotein (*DSPP*) gene expression (Shiba et al, 2003). In addition, stimulation of odontoblast-like cells with recombinant hBD-2 leads to increased mRNA expression of several inflammatory genes, including IL-6, IL-8, and cytosolic phospholipase A_2 (Dommisch et al, 2007). Consequently, it is probable that hBD-2 plays a role in reparative dentin formation, as well as immune regulation, in addition to its antimicrobial effect. Furthermore, like LL-37, hBD-2, hBD-3, and hBD-4 stimulate cell migration and proliferation, and production of cytokines and chemokines in skin keratinocytes (Niyonsaba et al, 2007).

Disease Implications

Several studies have shown the association between altered expression of epithelial-derived antimicrobial peptides, including LL-37 and humanβ-defensins, and various skin and epithelial diseases, e.g., acne vulgaris (Chronnell et al, 2001), oral lichen planus, leukoplakia (Nishimura et al, 2003), oral candidiasis (Abiko et al, 2002), condyloma acuminatum, verruca vulgaris (Conner et al, 2002), cholesteatoma (Jung et al, 2003), chronic nasal inflammatory disease (Kim et al, 2003), etc.

Due to space limitations, only one classic example of alteration in antimicrobial peptide expression is presented here to demonstrate the clinical significance of these antimicrobial

peptides in the pathogenesis of inflammatory skin diseases. This example is described in some studies related to two well-characterized skin diseases, psoriasis and atopic dermatitis. In psoriatic lesions, expression of LL-37, hBD-2, and hBD-3 is up-regulated (Frohm et al, 1997; Harder et al, 1997a; Harder et al, 2001), whereas expression of these three peptides is significantly reduced in atopic dermatitis lesions (Ong et al, 2002). The difference in the levels of antimicrobial peptide expression between psoriasis and atopic dermatitis can be elaborated by different cytokine milieus between these two skin diseases, a Th1 versus a Th2 profile, respectively (Nomura et al, 2003). It has been demonstrated that enhanced production of IL-4 and IL-13, two cytokines categorized as a Th2 profile, in atopic dermatitis, can block expression of some antimicrobial peptides in skin keratinocytes (Nomura et al, 2003), which may then account for the reduction of antimicrobial peptide expression in this lesion.

It is known that one basic function of human skin is to form a natural barrier against microbial colonization and invasion, which leads to tissue homeostasis. To further enhance this function, the skin can also produce several antimicrobial peptides, which help control the number and types of microorganisms on the skin. If the production of antimicrobial peptides is impaired by dysfunction of the host immune system as a result of the pathogenesis of skin diseases, an increased risk of opportunistic infections from bacteria or viruses in the skin lesion ensues. Consequently, the deficiency of antimicrobial peptides, particularly LL-37, in atopic dermatitis lesions causes frequent infections from vaccinia virus (Howell et al, 2004). Similarly, a drastic reduction of LL-37 protein expressionthat results in increased susceptibility to infections has also been observed in patients with acute myeloid leukemia (An et al, 2005).

With respect to periodontal disease, data regarding the expression of β-defensin antimicrobial peptides in different types of periodontal diseases compared to healthy periodontal tissue are still contradictory and inconclusive. For example, the findings from one study (Dommisch et al, 2005) showed no significant differences in β-defensin mRNA expression in different clinical stages of periodontal disease as compared to that in normal tissue. Nevertheless, in the same study, hBD-2 expression was found to be significantly higher than hBD-1 expression in both gingivitis and periodontitis groups (Dommisch et al, 2005). In contrast, it was later shown in another study (Vardar-Sengul et al, 2007) that the levels of hBD-1 expression did not significantly differ from those of hBD-2 expression in patients with gingivitis. However, in patients with periodontitis, hBD-1 expression was significantly higher than hBD-2 expression in chronic periodontitis, whereas hBD-2 expression was significantly higher than hBD-1 expression in aggressive periodontitis (Vardar-Sengul et al, 2007). The reason behind these discrepancies may be due to a small number of patients and healthy volunteers, recruited in each study. Consequently, before any conclusions can be drawn for the relationship between β-defensin expression and periodontal disease, a larger study is required for assessing more accurate levels of β-defensin expression in both healthy and diseased tissues, obtained from different types of periodontal diseases.

It is noteworthy that significant up-regulation of both hBD-1 and hBD-2 expression is found in periodontal pocket epithelium as compared to the adjacent healthy epithelium from the same patient (Lu et al, 2004). In contrast, higher levels of hBD-3 expression are found in periodontally healthy tissues as compared to diseased tissues (Bissell et al, 2004). These may suggest differential functions between hBD-1/hBD-2 and hBD-3 in periodontal disease, and

also a more protective role for hBD-3 in regulating host immune responses to microbial assaults, as mentioned under the previous headings.

To the best of our knowledge, there has been no report that shows the relationship between the deficiency of β-defensin expression in periodontal tissues and periodontal diseases. On the contrary, both LL-37, which is mainly derived from neutrophils, and neutrophilα-defensins show a direct link to the pathogenesis of a certain type of periodontitis. This is revealed by one study (Pütsep et al, 2002) that shows the deficiency in LL-37 and the reduction of neutrophil α-defensins in patients with morbus Kostmann syndrome, a severe congenital neutropenia. These patients suffer from recurrent gingivitis and even severe periodontitis during early childhood that result from the lack of neutrophil-derived antimicrobial peptides. Furthermore, it has been demonstrated *invitro* that several periodontal pathogens, e.g., *Aggregatibacter actinomycetemcomitans*, are sensitive to the bactericidal effects of LL-37 (Tanaka et al, 2000; Isogai et al, 2003), so it is likely that the defective antimicrobial function of neutrophils from patients with morbus Kostmann syndrome, who are deficient in LL-37, cannot eliminate *Aggregatibacter actinomycetemcomitams*, which is highly associated with early-onset periodontitis.

In this regard, it is interesting to further investigate whether the deficiency of these antimicrobial peptides is also implicated with other forms of periodontitis associated with a syndrome, for instance, juvenile periodontitis in Papillon-Lefèvre syndrome, whose abnormalities result from cathepsin C mutations (Hart et al, 1999; Toomes et al, 1999). Is it probable that some antimicrobial peptides are substrates for cathepsin C enzyme, and these peptides may become more active after enzymatic degradation? If the answer is positive, one can assume that impaired cathepsin C function may not yield sufficient amounts of active antimicrobial peptides to exert their antimicrobial effects on periodontal pathogens. The deficiency in active antimicrobial peptides finally leads to repeated periodontal infections.

Conclusions and Interesting Research Topics

Substantial variations in expression of small cationic antimicrobial peptides, including LL-37 and defensins, in periodontal tissues, GCF, and saliva, exist and may be correlated with the pathogenesis of periodontal disease, as well as that of other oral inflammatory and infectious diseases. Therefore, the association between altered expression of antimicrobial peptides and some types of periodontitis, specifically the ones that are associated with syndromes, should be further explored in detail. Moreover, expression of some antimicrobial peptides and their clinical significance in other oral diseases should be further studied. Perhaps, it is possible that some peptides could be further developed as biomarkers for diagnosis and/or prognosis of oral diseases in the future.

Up to now, accumulated data gathered from *in vitro* and *in vivo* studies have exhibited a broad range of antimicrobial activities against oral microorganisms, especially some periodontal pathogens in a planktonic state. With respect to these data, it is interesting to further examine the microbicidal effects of antimicrobial peptides on dental plaque microorganisms. In addition, it is now becoming increasingly evident that the functions of antimicrobial peptides are not restricted to their antimicrobial activities, as was initially thought. It is, therefore, likely that other novel, but undiscovered, functions of these peptides

will be unraveled in the near future. Consequently, additional studies into new biological activities of antimicrobial peptides are needed and will be beneficial for us to better understand and gain deep insight into the importance of these multifunctional molecules, particularly their essential roles in maintaining tissue homeostasis during the healthy and diseased states of the periodontium.

Furthermore, it is still necessary to continue regulation studies, involving cellular receptors and intracellular signaling pathways that mediate up-regulation of some inducible antimicrobial peptides, in order to understand the mechanisms used to enhance the expression of these peptides. In quest of new adjunctive treatment modalities against periodontitis, it is possible that enhancement of antimicrobial peptide expression by putative components of commensal periodontal bacteria that are not harmful to the humanbody, or by non-toxic agents, similar to vaccination, may be of significant interest in controlling the number of periodontopathic bacteria. Finally, we, as members of the health professions, should be constantly aware of the clinical significance of these antimicrobial peptides in the pathogenesis of oral infectious and inflammatory diseases, especially periodontal disease.

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References

Aarbiou, J., Ertmann, M., van Wetering, S., van Noort, P., Rook, D., Rabe, K.F., Litvinov, S.V., van Krieken, J.H., de Boer, W.I. & Hiemstra, P.S. (2002). *Human neutrophil defensins induce lung epithelial cell proliferationin vitro.* J Leukoc Biol 72(1):167-174.

Aarbiou, J., Verhoosel, R.M., van Wetering, S., de Boer, W.I., van Krieken, J.H., Litvinov, S.V., Rabe, K.F. & Hiemstra, P.S. (2004). *Neutrophil defensins enhance lung epithelial wound closure and mucin gene expression in vitro*. Am J Respir Cell Mol Biol 30(2):193-201.

Abiko, Y., Jinbu, Y., Noguchi, T., Nishimura, M., Kusano, K., Amaratunga, P., Shibata, T. & Kaku, T. (2002). *Upregulation of human beta-defensin 2 peptide expression in oral lichen planus, leukoplakia and candidiasis. An immunohistochemical study*. Pathol Res Pract 198(8):537-542.

Abiko, Y., Saitoh, M., Nishimura, M., Yamazaki, M., Sawamura, D. & Kaku, T. (2007). *Role of beta-defensins in oral epithelial health and disease.* Med Mol Morphol 40(4):179-184.

Agerberth, B., Charo, J., Werr, J., Olsson, B., Idali, F., Lindbom, L., Kiessling, R., Jörnvall, H., Wigzell, H. & Gudmundsson, G.M. (2000). *The human antimicrobial and*

*chemotactic peptides LL-37 and alpha-defensins are expressed by specific lymphocyte and monocyte populations.*Blood 96(9):3086-3093.

Agerberth, B., Gunne, H., Oderberg, J., Kogner, P., Boman, H.G. & Gudmundsson, G.H. (1995*). Fall-39, a putative human peptide antibiotic, is cysteine-free and expressed in bone marrow and testis*. Proc Natl Acad Sci USA 92(1):195-199.

Ali, R.S., Falconer, A., Ikram, M., Bissett, C.E., Cerio, R. & Quinn, A.G. (2001). *Expression of the peptide antibiotics human beta defensin-1 and human beta defensin-2 in normal human skin*. JInvest Dermatol 117(1):106-111.

An, L.L., Ma, X.T., Yang, Y.H., Lin, Y.M., Song, Y.H. & Wu, K.F. (2005). *Marked reduction of LL-37/hCAP-18, an antimicrobial peptide, in patients with acute myeloid leukemia*. Int J Haematol 81(1):45-47.

Bachrach, G., Altman, H., Kolenbrander, P.E., Chalmers, N.I., Gabai-Gutner, M., Mor, A., Friedman, M. & Steinberg, D. (2008). *Resistance of Porphyromonas gingivalis ATCC33277 to direct killing by antimicrobial peptides is protease independent.* Antimicrob Agents Chemother 52(2):638-642.

Bals, R., Wang, X., Zasloff, M. & Wilson, J.M. (1998). *The peptide antibiotic LL-37/hCAP-18 is expressed in epithelia of the human lung where it has broad antimicrobial activity at the airway surface.*Proc Natl Acad Sci USA 95(16):9541-9546.

Becker, M.N., Diamond, G., Verghese, M.W. & Randell, S.H. (2000). *CD14-dependent lipopolysaccharide-induced beta-defensin-2 expression in human tracheobronchial epithelium.*J Biol Chem 275(38):29731-29736.

Befus, A.D., Mowat, C., Gilchrist, M., Hu, J., Solomon, S. & Bateman, A. (1999). *Neutrophil defensins induce histamine secretion from mast cells: mechanisms of action*. J Immunol 163(2):947-953.

Belas, R., Manos, J. & Suvanasuthi, R. (2004). *Proteus mirabilis ZapA metalloprotease degrades a broad spectrum of substrates, including antimicrobial peptides*. Infect Immun 72(9):5159-5167.

Bensch, K.W., Raida, M., Mägert, H.J., Schulz-Knappe, P. & Forssmann, W.G. (1995). *hBD-1: a novel beta-defensin from human plasma.*FEBS Lett 368(2):331-335.

Bevins, C.L., Jones, D.E., Dutra, A., Schaffzin, J. & Muenke, M. (1996). *Human enteric defensin genes: chromosomal map position and a model for possible evolutionary relationships.*Genomics 31(1):95-106.

Bissell, J., Joly, S., Johnson, G.K., Organ, C.C., Dawson, D., McCray, P.B.Jr. & Guthmiller, J.M. (2004*). Expression of beta-defensins in gingival health and in periodontal disease*. J Oral PatholMed 33(5):278-285.

Blomqvist, M., Bergquist, J., Westman, A., Hâkansson, K., Hâkansson, P., Fredman, P. & Ekman, R. (1999). *Identification of defensins in human lymphocyte nuclei*. Eur J Biochem 263(2):312-318.

Bollag, W.B., Zhong, X., Dodd, M.E., Hardy, D.M., Zheng, X. & Allred, W.T. (2005). *Phospholipase D signaling and extracellular signal-regulated kinase-1 and -2 phosphorylation (activation) are required for maximal phorbol ester-induced transglutaminase activity, a marker of keratinocyte differentiation*. J Pharmacol Exp Ther 312(3):1223-1231.

Bonass, W.A., High, A.S., Owen, P.J. & Devine, D.A. (1999). *Expression of beta-defensin genes by human salivary glands.*Oral Microbiol Immunol 14(6):371-374.

Bowdish, D.M., Davidson, D.J., Speert, D.P. & Hancock, R.E. (2004). *The human cationic peptide LL-37 induces activation of the extracellular signal-regulated kinase and p38 kinase pathways in primary human monocytes*. J Immunol 172(16):3758-3765.

Braff, M.H., Hawkins, M.A., Di Nardo, A., Lopez-Garcia, B., Howell, M.D., Wong, C., Lin, K., Streib, J.E., Dorschner, R., Leung, D.Y. & Gallo, R.L. (2005). *Structure-function relationships among human cathelicidin peptides: dissociation of antimicrobial properties from host immunostimulatory activities*. J Immunol 174(7):4271-4278.

Brissette, C.A.&Lukehart, S.A. (2002). *Treponema denticola is resistant to human β-defensins.*Infect Immun 70(7):3982-3984.

Brissette, C.A.&Lukehart, S.A. (2007). *Mechanisms of decreased susceptibility to beta-defensins by Treponema denticola.*Infect Immun 75(5):2307-2315.

Brissette, C.A., Pham, T.-T.T., Coats, S.R., Darveau, R.P.&Lukehart, S.A. (2008). *Treponema denticola does not induce production of common innate immune mediators from primary gingival epithelial cells*. Oral Microbiol Immunol 23(6):474-481.

Carlisle, M.D., Srikantha, R.N. & Brogden, K.A. (2009). *Degradation of α- and β-defensins by culture supernatants of Porphyromonas gingivalis strain 381*. J Innate Immun 1(2):118-122.

Carretero, M., Escámez, M.J., García, M., Duarte, B., Holguín, A., Retamosa, L., Jorcano, J.L., Río, M.D. & Larcher, F. (2008). *In vitro and in vivo wound healing-promoting activities of human cathelicidin LL-37*. J Invest Dermatol 128(1):223-236.

Chaly, Y.V., Paleolog, E.M., Kolesnikova, T.S., Tikhonov, I.I., Petratchenko, E.V. & Voitenok, N.N. (2000). *Neutrophil alpha-defensin human neutrophil peptide modulates cytokine production in human monocytes and adhesion molecule expression in endothelial cells*. Eur Cytokine Netw 11(2):257-266.

Chamorro, C.I., Weber, G., Grönberg, A., Pivarcsi, A. & Ståhle, M. (2009). *The human antimicrobial peptide LL-37 suppresses apoptosis in keratinocytes*. J Invest Dermatol 129(4):937-944.

Chertov, O., Michiel, D.F., Xu, L., Wang, J.M., Tani, K., Murphy, W.J., Longo, D.L., Taub, D.D. & Oppenheim, J.J. (1996). *Identification of defensin-1, defensin-2, and CAP37/ azurocidin as T cell chemoattractant proteins released from interleukin-8-stimulated neutrophils*. J Biol Chem 271(6):2935-2940.

Chotjumlong, P., Khongkhunthian, S., Ongchai, S., Reutrakul, V. & Krisanaprakornkit, S. (2010). *Human β-defensin-3 up-regulates cyclooxygenase-2 expression and prostaglandin E_2 synthesis in human gingival fibroblasts*. J Periodontal Res (doi:10.1111/ j.1600-0765.2009.01259.x).

Chronnell, C.M., Ghali, L.R., Ali, R.S., Quinn, A.G., Holland, D.B., Bull, J.J., Cunliffe, W.J., McKay, I.A., Philpott, M.P. & Müller-Röver, S. (2001). *Human beta defensin-1 and -2 expression in human pilosebaceous units: upregulation in acne vulgaris lesions*. J Invest Dermatol 117(5):1120-1125.

Chung, W.O., Hansen, S.R., Rao, D. & Dale, B.A. (2004). *Protease-activated receptor signaling increases epithelial antimicrobial peptide expression*. J Immunol 173(8):5165-5170.

Ciornei, C.D., Egesten, A. & Bodelsson, M. (2003). *Effects of human cathelicidin antimicrobial peptide LL-37 on lipopolysaccharide-induced nitric oxide release from rat aorta in vitro*. Acta Anaesthesiol Scand 47(2):213-220.

Conner, K., Nern, K., Rudisill, J., O'Grady, T. & Gallo, R.L. (2002). *The antimicrobial peptide LL-37 is expressed by keratinocytes in condyloma acuminatum and verruca vulgaris*. J Am Acad Dermatol 47(3):347-350.

Cowland, J.B., Johnsen, A.H. & Borregaard, N. (1995). *hCAP-18, a cathelin/pro-bactenecin-like protein of human neutrophil specific granules.*FEBS Lett 368(1):173-176.

Dale, B.A. & Fredericks, L.P. (2005). *Antimicrobial peptides in the oral environment: expression and function in health and disease*. Curr Issues Mol Biol 7(2):119-133.

Dale, B.A., Kimball, J.R., Krisanaprakornkit, S., Roberts, F., Robinovitch, M., O'Neal, R., Valore, E.V., Ganz, T., Anderson, G.M. & Weinberg, A. (2001). *Localized antimicrobial peptide expression in human gingiva.*J Periodontal Res 36(5):285-294.

Darveau, R.P., Belton, C.M., Reife, R.A. & Lamont, R.J. (1998). *Local chemokine paralysis, a novel pathogenic mechanism for Porphyromonas gingivalis.*Infect Immun 66(4):1660-1665.

Davidson, D.J., Currie, A.J., Reid, G.S., Bowdish, D.M., MacDonald, K.L., Ma, R.C., Hancock, R.E. & Speert, D.P. (2004). *The cationic antimicrobial peptide LL-37 modulates dendritic cell differentiation and dendritic cell-induced T cell polarization*. J Immunol 172(2):1146-1156.

De Yang, Chen, Q., Schmidt, A.P., Anderson, G.M., Wang, J.M., Wooters, J., *Oppenheim, J.J. & Chertov, O. (2000). LL-37, the neutrophil granule- and epithelial cell-derived cathelicidin, utilizes formyl peptide receptor-like 1 (FPRL1) as a receptor to chemoattract human peripheral blood neutrophils, monocytes, and T cells.*J Exp Med 192 (7):1069-1074.

den Hertog, A.L., van Marle, J., van Veen, H.A., van't Hof, W., Bolscher, J.G., Veerman, E.C. & Nieuw Amerongen, A.V. (2005). *Candidacidal effects of two antimicrobial peptides: Histatin 5 causes small membrane defects, but LL-37 causes massive disruption of the cell membrane.*Biochem J 388(Pt 2):689-695.

Di Nardo, A., Vitiello, A. & Gallo, R.L. (2003). *Cutting edge: mast cell antimicrobial activity is mediated by expression of cathelicidin antimicrobial peptide*. J Immunol 170(5):2274-2278.

Diamond, D.L., Kimball, J.R., Krisanaprakornkit, S., Ganz, T. & Dale, B.A. (2001). *Detection of beta-defensins secreted by human oral epithelial cells*. J Immunol Methods 256(1-2):65-76.

Dommisch, H., Açil, Y., Dunsche, A., Winter, J. & Jepsen, S. (2005). *Differential gene expression of human beta-defensins (hBD-1, -2, -3) in inflammatory gingival disease*. Oral MicrobiolImmunol 20(3):186-190.

Dommisch, H., Winter, J., Willebrand, C., Eberhard, J. & Jepsen, S. (2007). *Immune regulatory functions of human beta-defensin-2 in odontoblast-like cells.*Int Endod J 40(4):300-307.

Dunsche, A., Açil, Y., Siebert, R., Harder, J., Schröder, J.M. & Jepsen, S. (2001). *Expression profile of human defensins and antimicrobial proteins in oral tissues.*J Oral Pathol Med 30(3):154-158.

Dunsche, A., Açil, Y., Dommisch, H., Siebert, R., Schröder, J.M. & Jepsen, S. (2002). *The novel human beta-defensin-3 is widely expressed in oral tissues.*Eur J Oral Sci 110 (2):121-124.

Edfeldt, K., Agerberth, B., Rottenberg, M.E., Gudmundsson, G.H., Wang, X.B., Mandal, K., Xu, Q. & Yan, Z.Q. (2006*). Involvement of the antimicrobial peptide LL-37 in human atherosclerosis.*Arterioscler Thromb Vasc Biol 26(7):1551-1557.

Exton, J.H. (1999). *Regulation of phospholipase D*. Biochim Biophys Acta 1439(2):121-133.

Feucht, E.C., DeSanti, C.L. & Weinberg, A. (2003). *Selective induction of human beta-defensin mRNAs by Actinobacillus actinomycetemcomitans in primary and immortalized oral epithelial cells*. Oral Microbiol Immunol 18(6):359-363.

Filewod, N.C., Pistolic, J. & Hancock, R.E. (2009). *Low concentrations of LL-37 alter IL-8 production by keratinocytes and bronchial epithelial cells in response to pro-inflammatory stimuli.*FEMS Immunol Med Microbiol 56(3):233-240.

Frohm, M., Agerberth, B., Ahangari, G., Stâhle-Bäckdahl, M., Lidén, S., Wigzell, H. & Gudmundsson, G.H. (1997). *The expression of the gene coding for the antibacterial peptide LL-37 is induced in human keratinocytes during inflammatory disorders*. J Biol Chem 272(24):15258-15263.

Frohm Nilsson, M., Sandstedt, B., Sørensen, O., Weber, G., Borregaard, N. & Ståhle-Bäckdahl, M. (1999). *The human cationic antimicrobial protein (hCAP-18), a peptide antibiotic, is widely expressed in human squamous epithelia and colocalizes with interleukin-6.* Infect Immun 67(5):2561-2566.

Frye, M., Bargon, J. & Gropp, R. (2001). *Expression of human beta-defensin-1 promotes differentiation of keratinocytes*. J Mol Med 79(5-6):275-282.

Fukumoto, K., Nagaoka, I., Yamataka, A., Kobayashi, H., Yanai, T., Kato, Y. & Miyano, T. (2005). *Effect of antibacterial cathelicidin peptideCAP18/LL-37 on sepsis in neonatal rats*. Pediatr Surg Int 21(1):20-24.

Ganz, T., Selsted, M.E., Szklarek, D., Harwig, S.S., Daher, K., Bainton, D.F.&Lehrer, R.I. (1985). *Defensins, natural peptide antibiotics of human neutrophils*. J Clin Invest 76(4):1427-1435.

Ganz, T. (2003). *Defensins: antimicrobial peptides of innate immunity*. Nat Rev Immunol 3(9): 710-720.

García, J.R., Jaumann, F., Schulz, S., Krause, A., Rodríguez-Jiménez, J., Forssmann, U., Adermann, K., Klüver, E., Vogelmeier, C., Becker, D., Hedrich, R., Forssmann, W.G. & Bals, R. (2001). *Identification of a novel, multifunctional beta-defensin (human beta-defensin 3) with specific antimicrobial activity. Its interaction with plasma membranes of Xenopus oocytes and the induction of macrophage chemoattraction*. Cell Tissue Res 306(2):257-264.

Ghosh, D., Porter, E., Shen, B., Lee, S.K., Wilk, D., Drazba, J., Yadav, S.P., Crabb, J.W., Ganz, T. & Bevins, C.L. (2002). *Paneth cell trypsin is the processing enzyme for human defensin-5.*NatImmunol 3(6):583-590.

Goldman, M.J., Anderson, G.M., Stolzenberg, E.D., Kari, U.P., Zasloff, M. & Wilson, J.M. (1997). *Human beta defensin 1 is a salt sensitive antibiotic in lung that is inactivated in cystic fibrosis*. Cell 88(4):553-560.

Grigat, J., Soruri, A., Forssmann, U., Riggert, J. & Zwirner, J. (2007*). Chemoattraction of macrophages, T lymphocytes, and mast cells is evolutionarily conserved within the human α-defensin family.*J Immunol 179(6):3958-3965.

Han, W., Wang, W., Mohammed, K.A. & Su, Y. (2009). *α-defensins increase lung fibroblast proliferation and collagen synthesis via the β-catenin signaling pathway*. FEBS J 276(22):6603-6614.

Harder, J., Bartels, J., Christophers, E. & Schröder, J.M. (1997a). *A peptide antibiotic from human skin.* Nature 387(6636):861.

Harder, J., Siebert, R., Zhang, Y., Matthiesen, P., Christophers, E., Schlegelberger, B. & Schröder, J.M. (1997b). *Mapping of the gene encoding human beta-defensin-2 (DEFB2) to chromosome region 8p22-p23.1*.Genomics 46(3):472-475.

Harder, J., Meyer-Hoffert, U., Teran, L.M., Schwichtenberg, L., Bartels, J., Maune, S. & Schröder, J.M. (2000). *Mucoid Pseudomonas aeruginosa, TNF-alpha, and IL-1beta, but not IL-6, induce human beta-defensin-2 in respiratory epithelia*. Am J Respir Cell Mol Biol 22(6):714-721.

Harder, J., Bartels, J., Christophers, E. & Schröder, J.M. (2001). *Isolation and characterization of human beta-defensin-3, a novel human inducible peptide antibiotic*. J Biol Chem 276(8):5707-5713.

Hart, T.C., Hart, P.S., Bowden, D.W., Michalec, M.D., Callison, S.A., Walker, S.J., Zhang, Y. & Firatli, E. (1999). *Mutations of the cathepsin C gene are responsible for Papillon-Lefèvre syndrome*. J Med Genet 36(12):881-887.

Hase, K., Murakami, M., Iimura, M., Cole, S.P., Horibe, Y., Ohtake, T., Obonyo, M., Gallo, R.L., Eckmann, L. & Kagnoff, M.F. (2003). *Expression of LL-37 by human gastric epithelial cells as a potential host defense mechanism against Helicobacter pyroli.*Gastroenterology 125(6):1613-1625.

Heilborn, J.D., Nilsson, M.F., Kratz, G., Weber, G., Sørensen, O., Borregaard, N. & Ståhle-Bäckdahl, M. (2003). *The cathelicidin anti-microbial peptide LL-37 is involved in re-epithelialization of human skin wounds and is lacking in chronic ulcer epithelium*. J Invest Dermatol 120(3):379-389.

Hill, C.P., Yee, J., Selsted, M.E.& Eisenberg, D. (1991). *Crystal structure of defensin HNP-3, an amphiphilic dimer: mechanisms of membrane permeabilization*. Science 251(5000):1481-1485.

Hosokawa, I., Hosokawa ,Y., Komatsuzawa, H., Goncalves, R.B., Karimbux, N., Napimoga, M.H., Seki, M., Ouhara, K., Sugai, M., Taubman, M.A. & Kawai, T. (2006). *Innate immune peptide LL-37 displays distinct expression pattern from beta-defensins in inflamed gingival tissue*. Clin Exp Immunol 146(2):218-225.

Howell, M.D., Jones, J.F., Kisich, K.O., Streib, J.E., Gallo, R.L. & Leung, D.Y. (2004). *Selective killing of vaccinia virus by LL-37: implications for eczema vaccinatum*. J Immunol 172(3):1763-1767.

Huang, L.C., Petkova, T.D., Reins, R.Y., Proske, R.J. & McDermott, A.M. (2006). *Multifunctional roles of human cathelicidin (LL-37) at the ocular surface*. Invest Ophthalmol Vis Sci 47(6):2369-2380.

Isogai, E., Isogai, H., Matuo, K., Hirose, K., Kowashi, Y., Okumuara, K. & Hirata, M. (2003). *Sensitivity of genera Porphyromonas and Prevotella to the bactericidal action of C-terminal domain of humanCAP18 and its analogues*. Oral Microbiol Immunol 18(5):329-332.

Ji, S., Kim, Y., Min, B.-M., Han, S.H. & Choi, Y. (2007a). *Innate immune responses of gingival epithelial cells to nonperiodontopathic and periodontopathic bacteria*. J Periodontal Res 42(6):503-510.

Ji, S., Hyun, J., Park, E., Lee, B.-L., Kim, K.-K. & Choi, Y. (2007b). *Susceptibility of various oral bacteria to antimicrobial peptides and to phagocytosis by neutrophils.*J Periodontal Res 42(5):410-419.

Jia, H.P., Schutte, B.C., Schudy, A., Linzmeier, R., Guthmiller, J.M., Johnson, G.K., Tack, B.F., Mitros, J.P., Rosenthal, A., Ganz, T. & McCray, P.B.Jr. (2001). *Discovery of new human beta-defensins using a genomics-based approach*. Gene 263(1-2):211-218.

Joly, S., Maze, C., McCray, P.B.Jr. & Guthmiller, J.M. (2004). *Human β-defensin-2 and -3 demonstrate strain-selective activity against oral microorganisms.*J Clin Microbiol 42(3):1024-1029.

Joly, S., Organ, C.C., Johnson, G.K., McCray, P.B.Jr. & Guthmiller, J.M. (2005). *Correlation between β-defensin expression and induction profiles in gingival keratinocytes.*Mol Immunol 42(9):1073-1084.

Jones, D.E. & Bevins, C.L. (1992). *Paneth cells of the human small intestine express an antimicrobial peptide gene.*J Biol Chem 267(32):23216-23225.

Jones, D.E. & Bevins, C.L. (1993). *Defensin-6 mRNA in human Paneth cells: implications for antimicrobial peptides in the host defense of the human bowel*. FEBS Lett 315(2):187-192.

Jung, H.H., Chae, S.W., Jung, S.K., Kim, S.T., Lee, H.M. & Hwang, S.J. (2003). *Expression of a cathelicidin antimicrobial peptide is augmented in cholesteatoma*. Laryngoscope 113(3):432-435.

Karlsson, C., Andersson, M.L., Collin, M., Schmidtchen, A., Björck, L. & Frick, I.M. (2007). *SufA--a novel subtilisin-like serine proteinase of Finegoldia magna*. Microbiology 153(Pt12):4208-4218.

Kim, S.T., Cha, H.E., Kim, D.Y., Han, G.C., Chung, Y.S., Lee, Y.J., Hwang, Y.J. & Lee, H.M. (2003). *Antimicrobial peptide LL-37 is upregulated in chronic nasal inflammatory disease*. Acta Otolaryngol 123(1):81-85.

Koczulla, R., von Degenfeld, G., Kupatt, C., Krötz, F., Zahler, S., Gloe, T., Issbrücker, K., Unterberger, P., Zaiou, M., Lebherz, C., Karl, A., Raake, P., Pfosser, A., Boekstegers, P., Welsch, U., Hiemstra, P.S., Vogelmeier, C., Gallo, R.L., Clauss, M. & Bals, R. (2003). *An angiogenic role for the human peptide antibiotic LL-37/hCAP-18*. J Clin Invest 111(11):1665-1672.

Kopitar, M., Ritonja, A., Popovic, T., Gabrijelcic, D., Krizaj, I. & Turk, V. (1989). *A new type of low-molecular mass cysteine proteinase inhibitor from pig leukocytes.* Biol Chem Hoppe Seyler 370(10):1145-1151.

Krisanaprakornkit, S., Weinberg, A., Perez, C.N. & Dale, B.A. (1998). *Expression of the peptide antibiotic human beta-defensin 1 in cultured gingival epithelial cells and gingival tissue.*Infect Immun 66(9):4222-4228.

Krisanaprakornkit, S., Kimball, J.R., Weinberg, A., Darveau, R.P., Bainbridge, B.W. & Dale, B.A. (2000). *Inducible expression of human beta-defensin 2 by Fusobacterium nucleatum in oral epithelial cells: multiple signaling pathways and role of commensal bacteria in innate immunity and the epithelial barrier.*Infect Immun 68(5):2907-2915.

Krisanaprakornkit, S., Kimball, J.R. & Dale, B.A. (2002). *Regulation of human beta-defensin-2 in gingival epithelial cells: the involvement of mitogen-activated protein kinase pathways, but not the NF-kappaB transcription factor family*. J Immunol 168(1):316-324.

Krisanaprakornkit, S., Jotikasthira, D. & Dale, B.A. (2003). *Intracellular calcium in signaling humanβ-defensin-2 expression in oral epithelial cells*. J Dent Res 82(11):877-882.

Krisanaprakornkit, S., Chotjumlong, P., Kongtawelert, P. & Reutrakul, V. (2008). *Involvement of phospholipase D in regulating expression of anti-microbial peptide humanβ-defensin-2*. Int Immunol 20(1):21-29.

Lau, Y.E., Rozek, A., Scott, M.G., Goosney, D.L., Davidson, D.J. & Hancock, R.E. (2005). *Interaction and cellular localization of the human host defense peptide LL-37 with lung epithelial cells*. Infect Immun 73(1):583-591.

Laube, D.M., Dongari-Bagtzoglou, A., Kashleva, H., Eskdale, J., Gallagher, G. & Diamond, G. (2008). *Differential regulation of innate immune response genes in gingival epithelial cells stimulated with Aggregatibacter actinomycetemcomitans*.J Periodontal Res 43(1):116-123.

Liu, L., Zhao, C., Heng, H.H. & Ganz, T. (1997). *The human beta-defensin-1 and alpha-defensins are encoded by adjacent genes: two peptide families with differing disulfide topology share a common ancestry*. Genomics 43(3):316-320.

Liu, L., Wang, L., Jia, H.P., Zhao, C., Heng, H.H., Schutte, B.C., McCray, P.B.Jr. & Ganz, T. (1998). *Structure and mapping of the human beta-defensin HBD-2 gene and its expression at sites of inflammation*. Gene 222(2):237-244.

Lu, Q., Jin, L., Darveau, R.P. & Samaranayake, L.P. (2004). *Expression of human beta-defensin-1 and -2 peptides in unresolved chronic periodontitis*. J Periodontal Res 39(4):221-227.

Lu, Q., Samaranayake, L.P., Darveau, R.P. & Jin, L. (2005). *Expression of human beta-defensin-3 in gingival epithelia*.J Periodontal Res 40(6):474-481.

Lundy, F.T., Orr, D.F., Shaw, C., Lamey, P.-J. &Linden, G.J. (2005). *Detection of individual human neutrophilα-defensins (human neutrophil peptides 1, 2 and 3) in unfractionated gingival crevicular fluid-A MALDI-MS approach*. Mol Immunol 42(5):575-579.

Malm, J., Sørensen, O., Persson, T., Frohm-Nilsson, M., Johansson, B., Bjartell, A., Lilja, H., Ståhle-Bäckdahl, M., Borregaard, N. & Equesten, A. (2000). *The human cationic antimicrobial protein (hCAP-18) is expressed in the epithelium of the human epididymis, is present in seminal plasma at high concentrations, and is attached to spermatozoa*. Infect Immun 68(7):4297-4302.

Mars, W.M., Patmasiriwat, P., Maity, T., Huff, V., Weil, M.M. & Saunders, G.F. (1995). *Inheritance of unequal numbers of the genes encoding the human neutrophil defensins HP-1 and HP-3*.J BiolChem 270(51):30371-30376.

Mathews, M., Jia, H.P., Guthmiller, J.M., Losh, G., Graham, S., Johnson, G., Tack, B.F. & McCray, P.B.Jr. (1999). *Production of beta-defensin antimicrobial peptides by the oral mucosa and salivary glands*. Infect Immun 67(6):2740-2745.

McKay, M.S., Olson, E., Hesla, M.A., Panyutich, A., Ganz, T., Perkins, S. & Rossomando, E.F. (1999). *Immunomagnetic recovery of human neutrophil defensins from the human gingival crevice*. Oral Microbiol Immunol 14(3):190-193.

Midorikawa, K., Ouhara, K., Komatsuzawa, H., Kawai, T., Yamada, S., Fujiwara, T., Yamazaki, K., Sayama, K., Taubman, M.A., Kurihara, H., Hashimoto, K. & Sugai, M. (2003). *Staphylococcus aureus susceptibility to innate antimicrobial peptides, beta-defensins and CAP18, expressed by human keratinocytes*. Infect Immun 71(7):3730-3739.

Mizukawa, N., Sugiyama, K., Yamachika, E., Ueno, T., Mishima, K., Takagi, S. & Sugahara, T. (1999). *Presence of defensin in epithelial Langerhans cells adjacent to oral carcinoma and precancerous lesions*. Anticancer Res 19(4B):2969-2971.

Mizukawa, N., Sugiyama, K., Kamio, M., Yamachika, E., Ueno, T., Fukunaga, J., Takagi, S. & Sugahara, T. (2000). *Immunohistochemical staining of human alpha-defensin-1 (HNP-1), in the submandibular glands of patients with oral carcinomas*. Anticancer Res 20(2B):1125-1127.

Mookherjee, N., Brown, K.L., Bowdish, D.M., Doria, S., Falsafi, R., Hokamp, K., Roche, F.M., Mu, R., Doho, G.H., Pistolic, J., Powers, J.-P., Bryan, J., Brinkman, F.S. & Hancock, R.E. (2006). *Modulation of the TLR-mediated inflammatory response by the endogenous human host defense peptide LL-37*.J Immunol 176(4):2455-2464.

Murakami, M., Ohtake, T., Dorschner, R.A. & Gallo, R.L. (2002a). *Cathelicidin antimicrobial peptides are expressed in salivary glands and saliva.*J Dent Res 81(12):845-850.

Murakami, M., Ohtake, T., Dorschner, R.A., Schittek, B., Garbe, C. & Gallo, R.L. (2002b). *Cathelicidin antimicrobial peptide expression in sweat, an innate defense system for the skin.*J Invest Dermatol 119(5):1090-1095.

Murphy, C.J., Foster, B.A., Mannis, M.J., Selsted, M.E.& Reid, T.W. (1993). *Defensins are mitogenic for epithelial cells and fibroblasts.*J Cell Physiol 155(2):408-413.

Nagaoka, I., Hirota, S., Yomogida, S., Ohwada, A. & Hirata, M. (2000). *Synergistic actions of antibacterial neutrophil defensins and cathelicidins*. Inflamm Res 49(2):73-79.

Nassar, T., Akkawi, S., Bar-Shavit, R., Haj-Yehia, A., Bdeir, K., Al-Mehdi, A.B., Tarshis, M. & Higazi, A.A. (2002). *Human alpha-defensin regulates smooth muscle cell contraction: a role for low-density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor*. Blood 100(12):4026-4032.

Nishimura, E., Eto, A., Kato, M., Hashizume, S., Imai, S., Nisizawa, T. & Hanada, N. (2004). *Oral Streptococci exhibit diverse susceptibility to human beta-defensin-2: antimicrobial effects of hBD-2 on oral streptococci.*Curr Microbiol 48(2):85-87.

Nishimura, M., Abiko, Y., Kusano, K., Yamazaki, M., Saitoh, M., Mizoguchi, I., Jinbu, Y., Noguchi, T. & Kaku, T. (2003). *Localization of human beta-defensin 3 mRNA in normal oral epithelium, leukoplakia, and lichen planus: an in situ hybridization study.*Med Electron Microsc 36(2):94-97.

Niyonsaba, F., Someya, A., Hirata, M., Ogawa, H. & Nagaoka, I. (2001). *Evaluation of the effects of peptide antibiotics human beta-defensins-1/-2 and LL-37 on histamine release and prostaglandin D(2) production from mast cells*. Eur J Immunol 31(4):1066-1075.

Niyonsaba, F., Iwabuchi, K., Someya, A., Hirata, M., Matsuda, H., Ogawa, H. & Nagaoka, I. (2002a). *A cathelicidin family of human antibacterial peptide LL-37 induces mast cell chemotaxis.* Immunology 106(1):20-26.

Niyonsaba, F., Iwabuchi, K., Matsuda, H., Ogawa, H. & Nagaoka, I. (2002b). *Epithelial cell-derived human beta-defensin-2 acts as a chemotaxin for mast cells through a pertussis toxin-sensitive and phospholipase C-dependent pathway.*Int Immunol 14(4):421-426.

Niyonsaba, F., Ogawa, H. & Nagaoka, I. (2004). *Human beta-defensin-2 functions as a chemotactic agent for tumor necrosis factor-alpha-treated human neutrophils.* Immunology 111(3):273-281.

Niyonsaba, F., Ushio, H., Nakano, N., Ng, W., Sayama, K., Hashimoto, K., Nagaoka, I., Okumura, K. & Ogawa, H. (2007). *Antimicrobial peptides humanβ-defensins stimulate*

epidermal keratinocyte migration, proliferation, and production of proinflammatory cytokines and chemokines. J Invest Dermatol 127(3):594-604.

Noguchi, T., Shiba, H., Komatsuzawa, H., Mizuno, N., Uchida, Y., Ouhara, R., Asakawa, R., Kudo, S., Kawaguchi, H., Sugai, M. & Kurihara, H. (2003). *Synthesis of prostaglandin E_2 and E-cadherin and gene expression of β-defensin-2 by human gingival epithelial cells in response to Actinobacillus actinomycetemcomitans.*Inflammation 27(6):341-349.

Nomura, I., Goleva, E., Howell, M.D., Hamid, Q.A., Ong, P.Y., Hall, C.F., Darst, M.A., Gao, B., Boguniewicz, M., Travers, J.B. & Leung, D.Y. (2003). *Cytokine milieu of atopic dermatitis, as compared to psoriasis, skin prevents induction of innate immune response genes.*J Immunol 171(6):3262-3269.

Ohgami, K., Ilieva, I.B., Shiratori, K., Isogai, E., Yoshida, K., Kotake, S., Nishida, T., Mizuki, N. & Ohno, S. (2003). *Effect of human cationic antimicrobial protein 18 peptide on endotoxin-induced uveitis in rats.*Invest Ophthalmol Vis Sci 44(10):4412-4418.

Ong, P.Y., Ohtake, T., Brandt, C., Strickland, I., Boguniewicz, M., Ganz, T., Gallo, R.L. & Leung, D.Y. (2002). *Endogenous antimicrobial peptides and skin infections in atopic dermatitis.*N Engl J Med 347(15):1151-1160.

Oren, A., Ganz, T., Liu, L. & Meerloo, T. (2003). *In human epidermis, beta-defensin 2 is packaged in lamellar bodies.*Exp Mol Pathol 74(2):180-182.

Otte, J.M., Zdebik, A.E., Brand, S., Chromik, A.M., Strauss, S., Schmitz, F., Steinstraesser, L. & Schmidt, W.E. (2009). *Effects of the cathelicidin LL-37 on intestinal epithelial barrier integrity*. Regul Pept 156(1-3):104-117.

Ouhara, K., Komatsuzawa, H., Yamada, S., Shiba, H., Fujiwara, T., Ohara, M., Sayama, K., Hashimoto, K., Kurihara, H. & Sugai, M. (2005). *Susceptibilities of periodonto-pathogenic and cariogenic bacteria to antibacterial peptides, (beta)-defensins and LL-37, produced by human epithelial cells.*J Antimicrob Chemother 55(6):888-896.

Ouhara, K., Komatsuzawa, H., Shiba, H., Uchida, Y., Kawai, T., Sayama, K., Hashimoto, K., Taubman, M.A., Kurihara, H. & Sugai, M. (2006). *Actinobacillus actinomycetem-comitans outer membrane protein 100 triggers innate immunity and production of β-defensin and the 18-kilodalton cationic antimicrobial protein through the fibronectin-integrin pathway in human gingival epithelial cells.*Infect Immun 74(9):5211-5220.

Perregaux, D.G., Bhavsar, K., Contillo, L., Shi, J.&Gabel, C.A. (2002). *Antimicrobial peptides initiate IL-1 beta posttranslational processing: a novel role beyond innate immunity.* J Immunol 168(6):3024-3032.

Presicce, P., Giannelli, S., Taddeo, A., Villa, M.L. & Della Bella, S. (2009). *Human defensins activate monocyte-derived dendritic cells, promote the production of proinflammatory cytokines, and up-regulate the surface expression of CD91.* J Leukoc Biol 86(4):941-948.

Puklo, M., Guentsch, A., Hiemstra, P.S., Eick, S. & Potempa, J. (2008). *Analysis of neutrophil-derived antimicrobial peptides in gingival crevicular fluid suggests importance of cathelicidin LL-37 in the innate immune response against periodontogenic bacteria.*Oral Microbiol Immunol 23(4):328-335.

Pütsep, K., Carlsson, G., Boman, H.G. & Andersson, M. (2002). *Deficiency of antibacterial peptides in patients with morbus Kostmann: an observation study*. Lancet 360 (9340):1144-1149.

Ritonja, A., Kopitar, M., Jerala, R. & Turk, V. (1989). *Primary structure of a new cysteine proteinase inhibitor from pig leucocytes.*FEBS Lett 255(2):211-214.

Romeo, D., Skerlavaj, B., Bolognesi, M. & Gennaro, R. (1988). *Structure and bactericidal activity of an antibiotic dodecapeptide purified from bovine neutrophils*. J Biol Chem 263(20):9573-9575.

Sahasrabudhe, K.S., Kimball, J.R., Morton, T.H., Weinberg, A. & Dale, B.A. (2000). *Expression of the antimicrobial peptide, human beta-defensin 1, in duct cells of minor salivary glands and detection in saliva*. J Dent Res 79(9):1669-1674.

Schauber, J., Svanholm, C., Termén, S., Iffland, K., Menzel, T., Scheppach, W., Melcher, R., Agerberth, B., Lührs, H. & Gudmundsson, G.H. (2003). *Expression of the cathelicidin LL-37 is modulated by short chain fatty acids in colonocytes: relevance of signaling pathways.* Gut 52(5):735-741.

Schibli, D.J., Hunter, H.N., Aseyev, V., Starner, T.D., Wiencek, J.M., McCray, P.B.Jr., Tack, B.F. & Vogel, H.J. (2002). *The solution structure of the human beta-defensins lead to a better understanding of the potent bactericidal activity of HBD3 against Staphylococcus aureus.*J Biol Chem 277(10):8279-8289.

Schmidtchen, A., Frick, I.M., Andersson, E., Tapper, H. & Björck, L. (2002). *Proteinases of common pathogenic bacteria degrade and inactivate the antibacterial peptide LL-37*. MolMicrobiol 46(1):157-168.

Schnapp, D., Reid, C.J. & Harris, A. (1998). *Localization of expression of human beta defensin-1 in the pancreas and kidney*. J Pathol 186(1):99-103.

Schutte, B.C., Mitros, J.P., Bartlett, J.A., Walters, J.D., Jia, H.P., Welsh, M.J., Casavant, T.L. & McCray, P.B.Jr. (2002). *Discovery of five conserved beta-defensin gene clusters using a computational search strategy*. Proc NatlAcad Sci USA 99(4):2129-2133.

Scott, M.G., Davidson, D.J., Gold, M.R., Bowdish, D. & Hancock, R.E. (2002). *The human antimicrobial peptide LL-37 is a multifunctional modulator of innate immune responses.*J Immunol 169(7):3883-3891.

Semple, C.A., Rolfe, M. & Dorin, J.R. (2003). *Duplication and selection in the evolution of primate beta-defensin genes.* Genome Biol 4(5):R31.

Shaykhiev, R., Beisswenger, C., Kändler, K., Senske, J., Püchner, A., Damm, T., Behr, J. & Bals, R. (2005). *Human endogenous antibiotic LL-37 stimulates airway epithelial cell proliferation and wound closure.*Am J Physiol Lung Cell Mol Physiol 289(5):L842-L848.

Shiba, H., Mouri, Y., Komatsuzawa, H., Ouhara, K., Takeda, K., Sugai, M., Kinane, D.F. & Kurihara, H. (2003). *Macrophage inflammatory protein-3alpha and beta-defensin-2 stimulate dentin sialophosphoprotein gene expression in human pulp cells.*Biochem Biophys Res Commun 306(4):867-871.

Sørensen, O., Cowland, J.B., Askaa, J. & Borregaard, N. (1997). *An ELISA for hCAP-18, the cathelicidin present in human neutrophils and plasma*. J Immunol Methods 206(1-2): 53-59.

Sørensen, O.E., Follin, P., Johnsen, A.H., Calafat, J., Tjabringa, G.S., Hiemstra, P.S. & Borregaard, N. (2001). *Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3*. Blood 97(12):3951-3959.

Sørensen, O.E., Cowland, J.B., Theilgaard-Mönch, K., Liu, L., Ganz, T. & Borregaard, N. (2003). *Wound healing and expression of antimicrobial peptides/polypeptides in human keratinocytes, a consequence of common growth factors*. J Immunol 170(11):5583-5589.

Sparkes, R.S., Kronenberg, M., Heinzmann, C., Daher, K.A., Klisak, I., Ganz, T. & Mohandas, T. (1989). *Assignment of defensin gene(s) to human chromosome 8p23.* Genomics 5(2):240-244.

Taggart, C.C., Greene, C.M., Smith, S.G., Levine, R.L., McCray, P.B.Jr., O'Neill, S. & McElvaney, N.G. (2003). *Inactivation of human beta-defensin 2 and 3 by elastolytic cathepsins.*J Immunol 171(2):931-937.

Taguchi, Y. & Imai, H. (2006). *Expression of β-defensin-2 in human gingival epithelial cells in response to challenge with Porphyromonas gingivalis in vitro*. J Periodontal Res 41(4): 334-339.

Tanaka, D., Miyasaki, K.T.&Lehrer, R.I. (2000). *Sensitivity of Actinobacillus actinomycetemcomitans and Capnocytophaga spp. to the bactericidal action of LL-37: a cathelicidin found in human leukocytes and epithelium*. Oral Microbiol Immunol 15(4):226-231.

Tani, K., Murphy, W.J., Chertov, O., Salcedo, R., Koh, C.Y., Utsunomiya, I., Funakoshi, S., Asai, O., Herrmann, S.H., Wang, J.M., Kwak, L.W. & Oppenheim, J.J. (2000). *Defensins act as potent adjuvants that promote cellular and humoral immune responses in mice to a lymphoma idiotype and carrier antigens*. Int Immunol 12(5):691-700.

Tao, R., Jurevic, R.J., Coulton, K.K., Tsutsui, M.T., Roberts, M.C., Kimball, J.R., Wells, N., Berndt, J. & Dale, B.A. (2005). *Salivary antimicrobial peptide expression and dental cariesexperience in children.*Antimicrob Agents Chemother 49(9):3883-3888.

Taylor, K., Clarke, D.J., McCullough, B., Chin, W., Seo, E., De Yang, Oppenheim, J., Uhrin, D., Govan, J.R.W., Campopiano, D.J., MacMillan, D., Barran, P. & Dorin, J.R. (2008). *Analysis and separation of residues important for the chemoattractant and antimicrobial activities of β-defensin 3.*J Biol Chem 283(11):6631-6639.

Tjabringa, G.S., Aarbiou, J., Ninaber, D.K., Drijfhout, J.W., Sørensen, O.E., Borregaard, N., Rabe, K.F. & Hiemstra, P.S. (2003). *The antimicrobial peptide LL-37 activates innate immunity at the airway epithelial surface by transactivation of the epidermal growth factor receptor*. J Immunol 171(12):6690-6696.

Tjabringa, G.S., Ninaber, D.K., Drijfhout, J.W., Rabe, K.F. & Hiemstra, P.S. (2006). *Human cathelicidin LL-37 is a chemoattractant for eosinophils and neutrophils that acts via formyl-peptide receptors.* Int Arch Allergy Immunol 140(2):103-112.

Tokumaru, S., Sayama, K., Shirakata, Y., Komatsuzawa, H., Ouhara, K., Hanakawa, Y., Yahata, Y., Dai, X., Tohyama, M., Nagai, H., Yang, L., Higashiyama, S., Yoshimura, A., Sugai, M. & Hashimoto, K. (2005). *Induction of keratinocyte migration via transactivation of the epidermal growth factor receptor by the antimicrobial peptide LL-37*. J Immunol 175(7):4662-4668.

Tollin, M., Bergman, P., Svenberg, T., Jörnvall, H., Gudmundsson, G.H. & Agerberth, B. (2003). *Antimicrobial peptides in the first line defence of human colon mucosa*. Peptides 24(4): 523-530.

Toomes, C., James, J., Wood, A.J., Wu, C.L., McCormick, D., Lench, N., Hewitt, C., Moynihan, L., Roberts, E., Woods, C.G., Markham, A., Wong, M., Widmer, R., Ghaffar, K.A., Pemberton, M., Hussein, I.R., Temtamy, S.A., Davies, R., Read, A.P., Sloan, P., Dixon, M.J. & Thakker, N.S. (1999). *Loss-of-function mutations in the cathepsin C gene result in periodontal disease and palmoplantar keratosis*. Nat Genet 23(4):421-424.

Tsutsumi-Ishii, Y. & Nagaoka, I. (2002). *NF-kappa B-mediated transcriptional regulation of human beta-defensin-2 gene following lipopolysaccharide stimulation*. J Leukoc Biol 71(1):154-162.

Türkoğlu, O., Emingil, G., Kütükçüler, N. & Atilla, G. (2009). *Gingival crevicular fluid levels of cathelicidin LL-37 and interleukin-18 in patients with chronic periodontitis*. J Periodontol 80(6):969-976.

Valore, E.V. & Ganz, T. (1992). *Posttranslational processing of defensins in immature human myeloid cells*. Blood 79(6):1538-1544.

Valore, E.V., Park, C.H., Quayle, A.J., Wiles, K.R., McCray, P.B.Jr. & Ganz, T. (1998). *Human beta-defensin-1: an antimicrobial peptide of urogenital tissues*. J Clin Invest 101(8):1633-1642.

van Wetering, S., Mannesse-Lazeroms, S.P., van Sterkenburg, M.A., Daha, M.R., Dijkman, J.H. & Hiemstra, P.S. (1997). *Effect of defensins on interleukin-8 synthesis in airway epithelial cells.*Am J Physiol 272(5 Pt 1):L888-L896.

Vardar-Sengul, S., Demirci, T., Sen, B.H., Erkizan, V., Kurulgan, E. & Baylas, H. (2007). *Human beta defensin-1 and -2 expression in the gingiva of patients with specific periodontal diseases*. J Periodontal Res 42(5):429-437.

Weber, G., Heilborn, J.D., Chamorro Jimenez, C.I., Hammarsjo, A., Törmä, H. & Stahle, M. (2005). *Vitamin D induces the antimicrobial protein hCAP-18 in human skin*. J Invest Dermatol 124(5):1080-1082.

Wilde, C.G., Griffith, J.E., Marra, M.N., Snable, J.L. & Scott, R.W. (1989). *Purification and characterization of human neutrophil peptide 4, a novel member of the defensin family*. J Biol Chem 264(19):11200-11203.

Yang, D., Chertov, O., Bykovskaia, S.N., Chen, Q., Buffo, M.J., Shogan, J., Anderson, M., Schröder, J.M., Wang, J.M., Howard, O.M. & Oppenheim, J.J. (1999). *Beta-defensins: linking innate and adaptive immunity through dendritic and T cellCCR6*. Science 286(5439):525-528.

Yang, D., Chen, Q., Chertov, O. & Oppenheim, J.J. (2000). *Human neutrophil defensins selectively chemoattract naïve T and immature dendritic cells*. J Leukoc Biol 68(1):9-14.

Yu, J., Mookherjee, N., Wee, K., Bowdish, D.M., Pistolic, J., Li, Y., Rehaume, L. & Hancock, R.E. (2007). *Host defense peptide LL-37, in synergy with inflammatory mediator IL-1β, augments immune responses by multiple pathways*. J Immunol 179(11):7684-7691.

Zaiou, M., Nizet, V. & Gallo, R.L. (2003). *Antimicrobial and protease inhibitory functions of the human cathelicidin (hCAP-18/LL-37) prosequence*. J Invest Dermatol 120(5):810-816.

Zanetti, M., Gennaro, R. & Romeo, O. (1995). *Cathelicidins: a novel protein family with a common proregion and a variable C-terminal antimicrobial domain*. FEBS Lett 374(1):1-5.

Zasloff, M. (2002). *Antimicrobial peptides of multicellular organisms*. Nature 415(6870):389-395.

Zhao, C., Wang, I.&Lehrer, R.I. (1996). *Widespread expression of beta-defensin hBD-1 in human secretory glands and epithelial cells*. FEBS Lett 396(2-3):319-322.

Zuyderduyn, S., Ninaber, D.K., Hiemstra, P.S. & Rabe, K.F. (2006). *The antimicrobial peptide LL-37 enhances IL-8 release by human airway smooth muscle cells*. J Allergy Clin Immunol 117(6):1328-1335.

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